The influence of granular and liquid top-dressed nitrogen on nitrogen use efficiency (NUE), grain yield and quality parameters of spring wheat (*Triticum aestivum* L.)

by

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Abstract

Nitrogen use efficiency (NUE) of major cereal grains including wheat (*Triticum aestivum* L.) is estimated to be approximately 50% due to losses from leaching, soil denitrification, gaseous plant emissions, volatilization and surface runoff. Use of liquid nitrogen fertiliser to improve grain yields and quality parameters and N use efficiencies has demonstrated positive results; however, responses are inconsistent. Low N use efficiencies indicate the need to improve crop N recoveries and possible lower environmental pollution and the already high production costs. Studies on application of granular and liquid N topdressings to wheat are limited in South Africa.

Studies were conducted from 2013-2015 to evaluate the response of NUE, yield and quality parameters of spring wheat to granular (broadcast) and liquid (sprayed) N topdressings under field conditions at two locations (Roodebloem - 34° 13'31.55"S; 19° 26'13.76"E and Langgewens - 33° 16' 33.96" S; 18° 42' 14.4" E) of the Western Cape Province, and controlled glasshouse conditions (2013, 2014 and 2016, and 2014, 2015 and 2016) at the Department of Agronomy, University of Stellenbosch, South Africa. Following applications of N as limestone ammonium nitrate (LAN 28%) at 30 kg N ha^{-1} at sowing, granular [(LAN (28%), granular urea (46%)] and liquid [urea ammonium nitrate (UAN 32%), liquid urea (46%) solution] N topdressings (30 and 60 kg N ha^{-1}) were applied by means of single (tillering), and split (tillering and flowering) application on spring wheat.

The field study results showed that the interaction between locality and growing season significantly affected NUE and grain yields and Roodebloem showed significantly better responses in grain yield in two (2014 and 2015) of the three study years compared to Langgewens. The effect of N rate showed that higher mean grain yield was produced through the application of N at 60 kg ha^{-1} (3 920 kg ha^{-1}) compared to 30 kg ha^{-1} (3 577 kg ha^{-1}) at Langgewens in 2013. The N rate x method of N application interaction showed that grain yield was significantly improved by liquid N topdressing at 30 kg ha^{-1} compared to granular N at 30 kg N ha^{-1} and liquid N at 60 kg N ha^{-1}. Roodebloem (3 090 kg.ha^{-1}) produced significantly

higher mean grain yield compared to Langgewens (2 084 kg ha^{-1}). The protein content and falling number were not significantly affected by N treatment.

In the first glasshouse experiment, UAN applied at 60 kg N ha^{-1} significantly improved grain yield compared to other treatments. The responses showed that 60 kg N ha^{-1} promoted significantly higher yields and yield parameters compared to 30 kg N ha^{-1} and that liquid N topdressings were superior compared to granular applied N throughout the study.

Similarly, in the second glasshouse experiment, plant responses increased with increasing N rates. The method x timing interaction showed significant differences due to timing of N application for liquid N topdressings. Plants treated with liquid N once at tillering showed superior responses compared to split applications of liquid N.

NUE studies showed that different N use efficiency parameters were significantly improved by liquid N topdressings where the effects were significant both under field and glasshouse conditions. Seasonal rainfall was overall the main contributing source of variation in the studies conducted under field conditions .

Opsomming

Stikstofverbruikdoeltreffendheid (SVD) van die belangrikste graangewasse insluitend koring (*Triticum aestivum* L.) word geskat op ongeveer 50% as gevolg van verliese deur loging, grond denitrifikasie, afskeidings as gasse deur plante, vervlugtiging en oppervlakafloop. Die gebruik van stikstofbemesting in vloeibare vorm om graanopbrengste, kwaliteitsvlakke en stikstofverbruikdoeltreffendheid te verbeter, het positiewe resultate opgelewer maar die reaksies was wisselvallig. Lae stikstofverbruikdoeltreffendheid dui op die behoefte om stikstof (N) verhaling, omgewingsbesoedeling en hoë produksiekoste te verbeter. Baie min ondersoeke na toediening van N in korrelvorm en vloeistofvorm as kopbemesting is nog in Suid-Afrika uitgevoer.

Ondersoeke na die SVD, opbrengs en kwaliteitsveranderlikes van lentekoring in reaksie op toediening van N kopbemesting in korrelvorm (uitgestrooi) en vloeistofvorm (oorhoofs gespuit) is van 2013 tot 2015 uitgevoer onder droëlandtoestande op twee lokaliteite (Roodebloem - 34° 13’31.55”S; 19° 26’13.76”E en Langgewens - 33° 16' 33.96" S; 18° 42' 14.4" E) in die Wes Kaap Provinsie. Twee soortgelyke ondersoeke is onder beheerde glashuistoestande (2013, 2014 en 2016, en 2014, 2015 en 2016) by die Departement Agronomie, Universiteit van Stellenbosch, Suid-Afrika uitgevoer. Nadat kalksteen ammonium nitraat (KAN 28%) teen 30 kg N ha^{-1} met saai toegedien is, is N kopbemesting (30 en 60 kg N ha^{-1}) in korrelvorm [(KAN (28%), ureumkorrels (46%)] en vloeistofvorm [ureum ammonium nitraat (UAN 32%), opgeloste ureum (46%)] toegedien as enkeltoediening (stoelvorming) en gesplete toediening (stoelvorming en blomvorming) op lentekoring.

Die resultate van die veldproef het aangedui dat die interaksie tussen lokaliteit en groeiseisoen SVD en graanopbrengste betekenisvol beïnvloed het en Roodebloem het betekenisvol beter graanopbrengste as Langgewens gelewer in twee ((2014 en 2015) van die drie seisoene. Die invloed van N vlak het getoon dat hoër gemiddelde graanopbrengste verkry is deur toediening van 60 kg N ha^{-1} (3 920 kg ha^{-1}) vergeleke met 30 kg N ha^{-1} (3 577 kg ha^{-1}) op Langgewens in 2013. Die N vlak x N toedieningsmetode interaksie het aangedui dat graanopbrengs betekenisvol verbeter is deur toediening van vloeibare N kopbemesting teen 30 kg ha^{-1}

vergeleke met korrel N toediening teen 30 kg N ha^{-1} en vloeibare N toediening teen 60 kg N ha^{-1}. Roodebloem (3 090 kg ha^{-1}) het 'n betekenisvol hoër gemiddelde graanopbrengs getoon as Langgewens (2 084 kg ha^{-1}). Die proteieninhoud en valgetal is nie betekenisvol verhoog deur N behandelings nie.

In die eerste glashuisproef het UAN wat teen 60 kg N ha^{-1} toegedien is graanopbrengs betekenisvol verhoog vergeleke met ander behandelings. Die resultate het aangetoon dat 60 kg N ha^{-1} betekenisvolle beter graanopbrengste en oeskomponente tot gevolg gehad het vergeleke met 30 kg N ha^{-1} en dat vloeibare N kopbemesting beter presteer het as korrelvorms van N gedurende die verloop van die studie.

Soortgelyke resultate is in die tweede glashuisproef waargeneem waar plante se reaksie verbeter het met hoër N toedieningsvlakke. Die toedieningsmetode x toedieningstyd interaksie het betekenisvolle verskille aangetoon ten opsigte van tyd van N toediening vir vloeibare N kopbemestings. Plante wat een keer met vloeibare N bemesting behandel is het beter reaksies getoon as plante wat gesplete toedienings van dieselfde hoeveelheid N gekry het.

SVD ondersoeke het getoon dat verskillende SVD veranderlikes betekenisvol verbeter het met toediening van vloeibare N kopbemesting beide onder veldtoestande en in die glashuisproewe. Variasie in seisoenale reënval was die hoof bydraende faktor wat bygedra het tot die variasie wat in die veldproewe waargeneem is.

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List of abbreviations

AE – Agronomic efficiency

APE – Agrophysiological efficiency

AN – Ammonium nitrate

ANR – Apparent nutrient recovery

AS – Ammonium sulphate

CL – Cuticular layer

DAE – Days after emergence

DAP – Days after planting

EW – Epicutular wax

GY – Grain yield

GYPP – Grain yield per pot

HI – Harvest index

LAI – Leaf area index

LAN – Limestone ammonium nitrate

MEPP – Mass of ears per pot

NEPP – Number of ears per pot

N – Nitrogen

NHI – Nitrogen harvest index

NUE – Nitrogen use efficiency

PB – Plant biomass

PBPP – Plant biomass per pot

PFP_N – Partial factor productivity of nitrogen

PGPR – Plant growth-promoting *Rhizobacteria*

PE – Physiological efficiency

UAN – Urea ammonium nitrate

UAS – Urea ammonium sulphate

WUE – Water use efficiency

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Chapter 1

General introduction and study objectives

1.1 Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated and valuable food crops in the world (FAOSTAT 2010; Prasad and Hochmuch 2016). Yield and quality of produced grain play a critical role in the successful, economic production and marketing of wheat. Although yield was economically the most important factor to the producer, both yield and quality became the most important factors due to higher quality standards set out by the market (Otto 2007). Nitrogen (N) is one of the most important elements contributing to both yield and quality of the grain. However, nitrogen use efficiency (NUE) for cereal production (wheat, maize, rice, etc.) is around 33% with 67% being unaccounted for (Raun and Johnson 1999) and this is less than the 50% generally reported (Hardy and Havelka 1975). A review by Gupta and Khosla (2012) later reinstated the earlier reports of 50% NUE values reported by Hardy and Havelka (1975). The unaccounted losses indicate that billions of rands are lost annually on N fertilisers through gaseous plant emmission (Harper et al. 1987; Francis et al. 1993; Davidson 2009; Sainju 2017), soil denitrification (De Datta et al. 1991; Hilton et al. 1994; Prasad and Hochmuch 2016), surface runoff (Blevins et al. 1996), volatilization (Hargrove et al. 1977; Suddick et al. 2013; Shelton et al. 2018), and leaching (Olson and Swallow 1984; Raun and Johnson 1995; Kros et al. 2011). These losses in turn contribute to increased use of energy resources, higher production costs and possible environmental pollution due to leaching of nitrates to water resources (Sharpe et al. 1988; Semenov et al. 2007; Piccini et al. 2016).

As indicated in paragraph one above, there are several factors that contribute to lower NUEs of plants. However, Raun and Johnson (1999) suggested that some of the factors contributing to lower NUEs could be due to complacency by farmers and low fertiliser prices. In the United States of America (USA), N costs approximately \$0.49 kg^{-1} (R5.72 kg^{-1}) depending on the source of N. Addition of 40 kg N ha^{-1} at planting when average cereal N rates are greater than

100 kg N ha^{-1} will cost less than \$20 ha^{-1} (R239.60). Raun and Johnson (1999) reported that affordability and the convenience of only applying N once during the growing season is a popular practice amongst farmers. Schepers et al. (1991) concluded that application of excess N serves as insurance and is a result of optimism from farmers concerning expected yields and yield goals. The affordability of N in the developed world has led to the misuse and over-application of N. In contrast, the same cannot be said about the developing world, where access to fertiliser is limited (Hubbel 1995) especially to subsistence farmers in remote areas. Contrary to Raun and Johnson (1999), Holzapfel et al. (2007) reported that narrow profit margins and the rising price of N fertilisers has put producers under constant pressure to manage this nutrient more efficiently. DAFF (2016) reported that the demand for nitrogen fertilisers during the period between 2005 and 2015 increased because nitrogen-based fertilisers are the most used for crop production. According to Grain SA (2011), as production input, fertiliser contributes on average between 30 and 50% to a grain (wheat, maize etc.) and oilseed (canola, soybean etc.) producers' variable production costs in South Africa. Consequently, the price that grain and oilseed producers pay for fertiliser is a determining factor of the profitability of grain and oilseed production in South Africa. In addition to this statement, Cassman et al. (2002) reported that nitrogen fertilisers are one of the most expensive inputs in cereal cropping systems. This means that producers need to manage and efficiently use fertilisers in order to improve yields and nutrient use efficiencies at all times.

Raun and Johnson (1999) suggested that production practices that have resulted in improved NUE relative to conventional or standard practices are those that counter conditions or environments known to contribute to N loss from soil-plant systems. Amongst others, practices such as crop rotations, forage production systems, use of improved cultivars, conservation tillage, NH_4-N source, in-season and liquid (foliar-applied) N, irrigation and precision agriculture and application resolution have all shown some improvements in NUE. Liquid (foliar) application of nutrients to cereal crops is increasing, although it is still not a widely adopted practice (Fernándes et al. 2013). However, the results have been highly variable, showing significant benefit from liquid (foliar) applications at times, while on other occasions demonstrating no effect (Barraclough and Haynes 1996; Freeborn et al. 2001; Ma et al. 2004) and sometimes negative effects (Fageria et al. 2009). The reported negative effects are largely due to the foliar salts causing leaf scorch thus reducing the effective leaf area and the photosynthate production (Krogmeier et al. 1989; Gooding and Davies 1992; Phillips and Mullins 2004). Under windy conditions, it is difficult to apply liquid sprays due to nonuniform distribution of the nutrient solution, which may lead to variability in spray deposition (Fageria et al. 2009).

A review by Gooding and Davies (1992) indicated that there has been consistency in the report of positive benefits gained by using foliar urea to improve wheat protein content. Use of foliar urea to increase the protein content of the grain may also reduce the risks of nitrate leaching and denitrification (Gooding and Davies 1992) thereby improving NUE. Studies by Woolfolk et al. (2002) showed a significant linear increase in total grain N for both pre- and post-flowering applications of urea ammonium nitrate (UAN) in five out of six site-years. Raun and Zhang (2006) reported that foliar applications of UAN in wheat are highly recommended since NUE increased via foliar N absorption. However, if UAN is applied on days where temperatures exceed 21°C, severe leaf burn could be encountered, in addition to the ammonia N volatilization losses that significantly reduce efficiency. Amanullah et al. (2013) studied the response of maize (*Zea mays* L.) to foliar application of N (2%) from different sources and the application time. The authors concluded that late foliar N application, one week before tasseling up to silking, could increase productivity. In their study, foliar application of N increased plant height, 1000-grains weight, grains ear^{-1}, biomass and grain yield when compared to the control (water spray).

1.2 Rationale of this study

Contrasting results in the use of liquid fertiliser applications render a need to further research on this subject to close knowledge and information gaps. In some cases liquid (foliar) N application resulted in lower NUE (Angus and Fisher 1991) but in other cases, it significantly improved protein content of the wheat (Bly and Woodard 2003). Other results showed that the yield response to urea sprays have been variable and only increased yield under previous N limiting conditions (Abad et al. 2003). Du Plessis and Agenbag (1994) studied the effect of foliar (liquid) applied N and sulphur (S) on wheat growth, yields and baking quality. The authors found that increasing N fertilisation increased N uptake and N concentration of the plant and resulted in a higher S uptake. Increase in N fertilisation also improved the baking quality due to higher protein content of the grain and flour. Studies on comparisons of granular and liquid applied N fertiliser topdressings on NUE, grain yields and quality parameters of wheat are still limited in South Africa. The findings of this study have the potential to close information gaps particularly on this topic and could also challenge the local farmers to consider use of liquid N as an alternative to traditional methods (granular N application). Furthermore, the recent drought conditions experienced in the country including the small grain production areas of the country necessitates alternative methods of fertiliser management that can improve the uptake during the critical growth stages of the crop.

The main aim of this study was to evaluate the effect of granular [lime ammonium nitrate (LAN) and granular urea] and liquid (spray) applied (UAN and urea solutions) N fertiliser topdressings on NUE indices, grain yields, yield and quality parameters (protein content, falling number, 1000 kernel mass, hectolitre mass). Water use efficiency (WUE) of spring wheat under field conditions was also investigated.

1.3 Objectives of the study

To obtain possible answers in relation to the main aim of the study; the specific objectives of the study were as follows:

i) To determine the effect of LAN, urea (granular), urea (solution) and UAN (solution) topdressings on yields and quality parameters of spring wheat in the wheat producing areas of the Western Cape, South Africa.

ii) To determine the effect of LAN, urea (granular), urea (solution) and UAN topdressings on yield and quality parameters of spring wheat under controlled glasshouse conditions.

iii) To determine the effect of single and split applications of LAN, urea (granular), urea (solution) and UAN topdressings on yields and quality of spring wheat under controlled glasshouse conditions.

iv) To study the effect of granular and liquid applied N topdressings under field and glasshouse conditions on nitrogen use efficiency of spring wheat.

1.4 Dissertation outline

The research chapters of this study are presented as scientific publications, with Chapter 1 being the general introduction and objectives of the study. Chapter 2 reviews the literature with a strong emphasis on liquid application of N on cereal grains and NUE of grain crops. Chapter 3 gives a detailed overview on how the study was conducted. For this reason, there will be some unavoidable repetitions between Chapter 3 and the research chapters.

Chapters 4 to 7 are in sequence of objectives outlined in Section 1.3 above and are written with their own abstracts, introductions, methodology, results and discussions, and conclusions. Chapter 7 consists of three sections, where SECTION A reports and discusses the NUE of spring wheat under field conditions and SECTION B and C focusses on the results and discussions of the two glasshouse experiments and are presented as Glasshouse experiment 1 and Glasshouse experiment 2 respectively. Chapter 8 is the summary and general conclusions and describes areas of possible future research.

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Chapter 2

Literature review

2.1 Introduction

Amongst other cereal crops wheat (*Triticum spp*.) is one of the most important and valuable food crop for all humans (FAOSTAT 2007; GCARD 2012; Curtis and Halford 2014). This statement suggests that sustainable production of this crop remains a necessity as the world population is expected to reach around 9.7 billion by 2050 (FAO 2004; UN DESA 2015). The global food demand projections show that more grain ranging between 50 and 70% will be required by 2050 to provide food to these people. According to FAO (2004), about half of the world population depend on nitrogen (N) fertilizer for the production of food and the world use of fertilizer N is estimated around 83 million metric tons (Mt). This implies that effective and efficient utilization of N will continue to be a priority particularly for its use in the production of grains. Part of the challenge in modern agriculture is that farmers are not only required to improve yields of the grain crops but there is also a need to monitor the impact of production systems on the environment due to concern over the contribution of N fertilisers to environmental problems. These include nitrate pollution of waters and the pollution of the atmosphere with nitrous oxide and other oxides of N and ammonia (Sharpe et al. 1988; Byrnes 1990; Semenov et al. 2007). At farm level, nitrogen (N) is one of the nutrients that often limit the production potential of grain crops.

Aspects regarding N fertilization to improve N-use efficiency (NUE) in wheat production systems have been researched over the past decades through developing fertilizer management strategies that are based on N supply and crop N demand (Ladha et al. 2005). Amongst others, use of liquid fertilizers, which were applied on larger scale about 60 years ago are gaining popularity as one of the strategies involved in improving efficiency of N. Balasubramanian et al. (2004) reported that NUE may be improved with liquid (foliar) application of urea or ammonium nitrate solutions. The authors indicated that liquid (foliar-applied) N is less subject to surface run-off, microbial immobilization, volatilization, and denitrification compared to soil-applied N. Basic research on use of liquid (N-topdressings) N fertilisers on crops has been conducted but not many comparisons with granular soil applied fertilisers earlier in the season were made. Most research results report on the efficiency of foliar applied liquid fertilisers applied later in the season in improving wheat grain protein content. Information on comparisons between efficiency of foliar applied liquid N fertilisers and soil applied granular fertilisers in the early vegetative growth stages of grains is still scanty. This literature review will focus on but is not limited to use of liquid (N topdressings) applied fertilisers with a particular emphasis on their effects on yields, grain quality and NUE of main crop plants. The latest information on the use of liquid applied N fertilisers will be discussed and where possible, depending on the availability, data on comparisons between granular applied N fertilisers and liquid applied N fertilisers will also be discussed. Furthermore, other critical factors of N management within the plant-soil-environment continuum will be reviewed.

2.2 Advantages and disadvantages of liquid fertilisers

Compared to granular soil applications, liquid (foliar) applications enable plants to utilize the applied nutrients rapidly, which allows for the reversal of deficiencies in a short period of time (Fageria et al. 2009). It is generally accepted that crops take up to six days to respond to soil applied fertilisers if climatic conditions are favourable. In contrast, quicker crop responses have been reported in foliar application, which normally takes three to four days (Fageria et al. 2009). The advantage with soil applications is that they tend to have a lasting effect on plant, while growth responses to liquid sprays application show a short period (hours to few days). This suggests that under severe nutrient deficiency, several liquid (foliar sprays) applications are required (Fageria et al. 2009). Using liquid applications when plant roots are not well established improves nutrient absorption compared to granular application. However, Gooding and Davies (1992) reported that a minimum leaf area index (LAI) for the improvement of spray uptake is a prerequisite. Thorne (1955); Gooding and Davies (1992) reported that a LAI ranging between 2 and 4 is often sufficient in wheat. Fageria et al. (2009) indicated that there is a possibility of leaf burn if the concentration of the nutrient is higher than what the leaves can tolerate, which is generally not the case with soil application. Turley et al. (2001) reported that damage by leaf scorch could affect about 10% of the sprayed leaves at application rates of 40-60 kg N ha^{-1}.

Wind is known to be a significant factor contributing to differences in the uptake of liquid spray application. As a result, high pre-cautionary measures are necessary on a windy day to avoid non-uniform distribution of the nutrient solution (Fageria et al. 2009). These challenges are not common with granular soil applied fertilisers. Gooding and Davies (1992) reported that there are several advantages associated with feeding N to cereals through liquid (foliar) urea. These include limited N losses through leaching and denitrification compared to traditional N fertiliser applications. Secondly, liquid applied urea solution improves the ability to provide N when root activity is damaged, e.g. under dry conditions or late in the season to increase grain N concentration. A recent study by Hussain et al. (2016) found that a combined N and potassium (K) liquid spray significantly enhanced water relations, gas exchange characteristics and resulted in high antioxidant activity that ultimately increased the yield under water deficit conditions in sunflower (*Helianthus annuus* L.). Liquid application of N and K combined significantly improved nutrient uptake and accumulation in crop plants under water stress conditions.

According to Gooding et al. (1988) and Peltonen et al. (1991), foliar application of urea can reduce the occurrence of some diseases and this in turn may offer yield benefits. Furthermore, spraying of fertilisers provide a window of opportunity to add other agrochemicals in the same tank mixes resulting into reduced labour, farm machinery and energy cost (Gooding and Davies 1992). In wheat, liquid (foliar) application of N can increase grain protein content and the baking quality if the timing of application is appropriate compared to soil applied granular N. From the above, it is clear that there is a number of benefits associated with the use of liquid (foliar spray) application of fertilizers. More work is however, needed to further close the gaps that exist in the use of this technology.

2.3 Crop responses to liquid (foliar) applications

Most of the research activities reported on responses of crops to liquid (foliar) fertilisation were done on wheat and soybean (Fageria et al. 2009) and recently on potatoes (Qadri et al. 2015). The general assessment thus far suggests that liquid (foliar) applications have resulted in positive, sometimes negative responses, while no responses were reported at times depending on plant species and nutrient element in question.

In wheat, a liquid N spray application resulted in higher grain protein content compared to dry granular fertiliser at late growth stages (mid-flowering compared to boot stage) (Alkier et al. 1972). Bly and Woodard (2003) reported that nine (9) out 12 sites showed significantly improved grain protein content responses to liquid (foliar) N application. Findings by Varga and Svečnjak (2006) indicated that there is a potential benefit in use of late urea spraying for improved grain yields in winter wheat if previous N applications were suboptimal for maximum yield potential. These authors also found that after spraying urea, grain protein content and wet gluten content were consistent at both low and high basal N fertilization regardless of variations in grain yields and protein concentrations across years. Saleem et al. (2013) reported that N concentration and N uptake in wheat grain was increased by liquid (foliar) application of urea (between early tillering and booting stage) compared to granular N (soil application) at sowing due to efficient mobilization of N to grain after liquid (foliar) fertilisation. An integrated use of both soil (granular) and foliar (liquid) application of urea gave similar yield to soil applied urea at the time of sowing. These authors concluded that foliar application of urea (2%) at two leaves, booting and tillering stage or 3% spray of urea at tillering and booting stage along with soil application is economical and can contribute to the compensation of yield losses. Khan et al. (2009) found that foliar application of urea significantly increased plant height, spike length, number of grains spike^{-1}, 100 grain weight, biological yield, grain yield and N uptake of wheat. The authors added that application of 4% urea solution was the most effective for enhancing both the quantitative and qualitative traits when applied at tillering, stem elongation and boot stage. Sultana et al. (2017) reported that foliar N application increased grain protein content by up to 7% under irrigation but the authors also noted that foliar N did not significantly influence grain protein content under rainfed conditions.

Although literature suggests a mixture of responses to liquid (foliar applied) fertilisers, it is apparent that there are a number of benefits associated with the use of liquid fertilisers as shown above. Further research is needed to solidify evidence on the effect of liquid fertilisers particularly on grain yields. At farm level, farmers are often attracted to technologies that will improve crop yields and for that reason, further research on this aspect is very important. Bly and Woodard (2003) suggested that an increase in grain protein content with foliar fertilisation applied at late growth stages (post-anthesis) in wheat could prevent penalty fees resulting from selling grain with low protein content and possibly result in premium returned to the producer in favourable years.

2.4 Mechanisms involved in the uptake of foliar (liquid) applied nutrients

Evidence suggests that absorption of organic and inorganic materials can also occur through the surfaces of leaves (Franke 1967). Nutrient uptake by plant leaves is different to that of roots because cell walls are covered by a cuticle, which is not the case with the root structure. Jeffree (2006) reported that the epicuticular wax layer (EW), the cuticular proper (CP) and the cuticular layer (CL) are the integral components of the cuticle. According to Franke (1967), these cuticular membranes are permeable to both organic and inorganic ions and un-dissociated molecules. The penetration of ions is determined by the nature of the charge, absorbability and ion radius. Franke (1967) reported that the energy required for uptake could be achieved via the process of respiratory metabolism, or from photosynthesis. Middleton and Sanderson (1965) and Franke (1967) reported that a foliar-applied nutrient passes through the cuticular wax, the cuticle, the cell wall and lastly the membrane in that order. However, under certain circumstances, the nutrient passes through these various layers, and sometimes it may pass through the spaces between these layers (Dybing and Currier 1961). Work by Eichert et al. (1998); Eichert and Burkhardt (2001); Fernandez and Eichert (2009) has shown that ions can also be taken up via the leaf stomata. According to Burkhardt et al. (1999), when the stomata are open, foliar absorption is often easier.

Havlin et al. (2005) reported that absorption of N through the plant leaf material occurs through microscopic pores in the leaf cuticle, or through the stomata. Tan et al. (1999) found that the density of the stomata is greater on the underside of leaves and the uptake of N applied to the underside was taken up more rapidly than compared to N applied on the upper surface of leaves (Tan et al. 1999). Schönherr (1976) reported that the pores of the cuticle have a negative fixed charge, which has a tendency to repel nitrate-N because of the common negative charge. Bowman and Paul (1992) and Tan et al. (1999) on the other hand reported that, urea has no charge and has been shown to be more rapidly absorbed than ammonium or nitrate. After the application of N on plant foliage, absorption by the plant is rapid. Bowman and Paul (1989, 1990, 1992) studied different species of turf grass (*Poa pratensis*, tall fescue, creeping bentgrass, perennial ryegrass, respectively) and they reported that between 35 and 55% of the N applied (25 g N L^{-1} at 5 g N m^{-2}) to the foliage was absorbed within 2-3 days. Similarly, Dawar et al. (2012) showed that approximately 30-40% N was taken up from foliar applied urea within few hours of application in pot experiments with perennial ryegrass. Variation in absorption of foliar-applied N could be explained by many factors such as rate of N applied, and the amount of water used to carry the dissolved nutrient, plant species, and the conditions during the time of application.

2.5 Factors affecting spray mechanisms

Success of the absorption and translocation of the applied nutrient is governed by the properties of the spray solution (Fernándes et al. 2013). The process of absorption of leaf-applied solution is rather complex and remains unclear. However, the properties of the formulations are associated with chemical principles as well as the prevailing environmental conditions during the time of spray applications. This section will discuss the principal physico-chemical factors involved in the liquid (foliar) application of nutrient solutions

2.5.1 Concentration of the spray solution

The concentration gradient between the nutrient spray solutions and that found within the plant will always be high (Schönherr 2001). The process through which nutrient solution is absorbed is diffusion as a result of a concentration gradient between the solution and the plant surface. Studies by Schönherr (2001) on isolated cuticles found that higher penetration rates were a result of increased concentrations of several applied mineral elements and Zhang and Brown (1999a,b) reported similar findings with intact leaves. Fernándes et al. (2013), however, reported that there is still lack of full understanding on the relationship between concentration of the applied nutrient element and foliar penetration rates. The type of nutrient, plant species, plant age, nutritional status and weather conditions are some of the factors that should be considered when choosing a mineral solution for foliar application (Wittwer and Teubner 1959; Wojcik 2004; Kannan 2010) and these factors will be reduced by the need to avoid phyto-toxicity. In efforts to avoid phyto-toxicity, Wojcik 2004 and Kannan 2010 suggested that the concentration range of a nutrient solution for foliar application should be determined according to the type of nutrient element (e.g. macro- or micro-element), plant species, plant age, nutritional status and weather conditions.

2.5.2 Molecular weight

Fernándes et al. (2013) reported that the size of the nutrient molecule in a solution affect the rate of penetration of a foliar fertiliser due to the mechanism involved in cuticular absorption. Beyer et al. (2005) reported that water and solutes cross the cuticle in an aqueous continuum while Schönherr (2006) suggested that this was achieved through aqueous pores.

Schreiber and Schönherr (2009) reported that the process of cuticular permeability is size-selective and the penetration generally favour low-molecular weight molecules compared to high molecular weight compounds. In contrast, Eichert and Goldbach (2008) reported that cuticular penetration route of entry that may indicate that there is a stomatal pathway shows that the foliar uptake pathway is less size-selective than would be predicted.

2.5.3 Solubility

One of the most important requirements for foliar uptake is that the nutrient element should be dissolved or suspended before application (Fernándes et al. 2013). Liquid applied fertilisers contain active chemical ingredients such as salts, chelates or complexes of mineral nutrients. Additives can be used to alter the physical characteristics of a chemical compound in a specific solvent at a given temperature. The saturation concentration is the highest limit of the solubility of a substance in a solvent where adding more solute will not significantly affect the solution concentration. Fernández et al. (2013) reported that for diffusion to take place the applied nutrient element must be dissolved in water in order to improve foliar uptake. The authors concluded that water solubility is critical for foliar uptake because when the applied nutrient is dissolved in a liquid phase, this will enable the diffusion from the plant surface into the plant organs.

2.5.4 Electric charge

Salts dissociate into free ions when dissolved in water with the final solution being electrically neutral (Fernández et al. 2013). The degree with which anions and cations present in an aqueous solution will be hydrated is determined by the physical and chemical characteristics. At pH levels above three, plant cuticles are negatively charged (Schönherr and Huber 1977) and charges of cell walls correspond to dissociated acids (Grignon and Sentenac 1991). Uncharged or electron-charged nutrient elements and anions can penetrate plant leaves and their translocation in the apoplast is reported to be fairly easier compared with positively-charged complexes or cations. When considering the anions, this statement is in contrast with Schönherr (1976), who reported that the pores of the cuticle have a negative fixed charge, which may repel nitrate-N due to the common negative charge. However, the nature of the anions and cations within a solution must be taken into account when designing spray formulations as it tends to have a physiological significance (Fernández et al. 2013).

2.5.5 Solution pH

The pH of the spray solution is known to alter the penetration ability, however, there is no consistency in plant responses to the pH of the solution. As such pH alone is not a clear indicator of the penetration rate and nutrients applied and the plant species in question seem to also play a significant role (Fernández et al. 2013).

There is currently limited scientific work focusing on the pH of the nutrient spray solution applied to the foliage particularly with reference to cereals. Fernandez and Ebert (2005) and Fernandez et al. (2006) reported that pH values around 5 were efficient in iron (Fe) uptake in studies conducted on peaches (*Prunus persica* L.). Blanpied (1979) reported that the solution pH raging between 3.3 and 5.2 improved the uptake of calcium (Ca) by apple leaves. Cook and Boynton (1952) demonstrated the improved uptake of urea by apple leaves in the pH range between 5.4 and 6.6.

2.6 Nitrogen management

Nitrogen is regarded as the nutrient that often limit crop production in many agricultural areas of the world and efficient use of N is important to ensure economic sustainability of cropping systems (Cassman et al. 2002). Due to its dynamic nature and vulnerability to loss processes from soil-plant systems, it creates a unique and challenging environment for its efficient management (Fageria and Baligar 2005). These authors reported that the right sources, adequate rates, efficient methods and the right application time are instrumental in any fertilizer program and crop demand should be the basis of N applications. These factors are vital for improving NUE and sustainable crop production, concluded these authors.

2.6.1 Sources of N

Improving NUE is a challenge and is more difficult than any other fertiliser nutrient. Part of the challenge is that N mobility in soil-plant systems is high and variable. Furthermore, many sources of addition and loss pathways of N in soil-plant systems occur resulting in complications of N balance and N use by plants. Fageria and Baligar (2005) reported that nitrogen sources and method of application significantly influence N uptake efficiency in crop plants. Important factors that play a role in considering sources of N by growers are availability, economics, convenience in storage and handling, and the effectiveness of the carrier. Generally, urea and ammonium sulfate are the principal sources of N fertilisers. However, several N containing fertilizers are available in the market as shown in Table 2.1. In addition to this list, growers in the small grain production areas of the Western Cape province of South Africa also use limestone ammonium nitrate (LAN, N - 28%) (Calcium ammonium nitrate elsewhere in the world) to supply N to their crops and the choice is generally influenced by the factors highlighted above. Furthermore, urea ammonium nitrate (UAN, N - 32%) is another source of N fertiliser that has gained popularity in the agriculture industry over the years. However, the scale of its use in the grain crop industry in South Africa is unclear. Doyle (2013) conducted a cost comparison between some liquid and dry N fertilisers using urea prices as a benchmark (Table 2.2). The author revealed that in kg kg^{-1}, liquid forms of N are more expensive as they cost between 30 and 75% more per unit of N compared to soil applied granular N sources. However, when applied, the cost per unit N applied can be comparable depending on the rate of N applied. According to the growers, the improved handling characteristics enable a reduction in cost of operations, staff and better spray utilization. For some growers, the convenience and ability to effectively applying N without depending on rainfall or irrigation for their incorporation out-weighed the significant extra cost.

2.6.2 Method of application

Nitrogen fertilisers can either be broadcast or mixed into soil before crops are planted. Unfortunately, surface broadcasting of N fertilisers can entail large losses, particularly ammonia volatilization, from the system and reduce NUE (Randall et al. 1985; Mohanty et al. 1999). Fertilisers may also be applied in the rows below the seed at sowing or banded in the rows beside the seed at planting or pre-emergence (Fageria and Baligar 2005). At post-emergence, fertilisers may be side-dressed by injecting them into sub-surface and top-dressed. The type of machinery available at the grower’s farm will influence which method is used more. Application of N fertilisers as liquid sprays is another method available for applying N fertilisers due to the benefits associated with this method. Liquid fertiliser application is not regarded as a substitute for soil application but can be considered as a supplement to soil applied granular fertilisers when soils are very sandy, alkaline, acidic, or waterlogged. This technique can also be used when quick recovery from N deficiency is required or under dryland farming areas where soil moisture is a constraint, or even when spraying does not involve an additional operation or expense (Ladha et al. 2005). Nitrogen recovery could be improved with liquid foliar sprays compared to any other method of application but their practical use is limited by the small amounts that can be applied, forcing frequent applications, and by the cost of equipment and labour (Novoa and Loomis 1981).

Table 2.1: Major nitrogen fertilisers

Common name	**Formula**	**N (%)**
Ammonium sulfate	$(NO_4)_2SO4$	21
Urea	$CO(NH_2)_2$	46
Urea ammonium nitrate	$CO(NH_2)_2 + NH_4NO_3$	32
Anhydrous ammonia	NH_3	82
Ammonium chloride	NH_4Cl	26
Ammonium nitrate	NH_4NO_3	35
Potassium nitrate	KNO_3	14
Sodium nitrate	$NaNO_3$	16
Calcium nitrate	$Ca(NO_3)_2$	16
Calcium cyanamide	$CaCN_2$	21
Ammonium nitrate sulfate	$NH_4NO_3(NH_4)_2SO_4$	26
Nitrochalk	$NH_4NO_3 + CaCO_3$	21
Monoammonium phosphate	NH_4HPO_4	11
Diammonium phosphate	$(NH_4)_2HPO_4$	18

Sources: Foth and Ellis (1988),Mengel et al. (2001)

Table 2.1: Cost comparison of different N sources when applied at 30 kg ha^{-1} (Cost presented in Australian dollars, 1 $ = R9.68)

Product	**UAN**	**AN Solution**	**Urea**	**Urea**	**Urea Solution**	**UAS**
Application	Dribble Band (Liquid)	Dribble Band (Liquid)	Banded Planter (Solid)	Ground Spread (Solid)	Foliar (Liquid)	Foliar (Liquid)
Analysis (N)	42%	25%	46%	46%	24%	27%
Delivered Cost (L or kg)	$0.95	$0.63	$0.59	$0.59	$0.45	$0.55
kg N to be applied	**30**	**30**	**30**	**30**	**30**	**30**
Rate ha^{-1} (kg or L)	71	120	65	65	125	111
$ kg^{-1} N	2.26	2.52	1.28	1.28	1.88	2.04
Product cost $ ha^{-1}	67.86	75.6	38.48	38.48	56.25	61.11
Application cost $ ha^{-1}	9	10	36	16	10	10
Total cost ($ Ha^{-1})	76.86 (R744.00)	85.6 (R828.61)	74.48 (R720.97)	54.48 (R527.37)	66.25 (R641.30)	71.11 (R688.34)

AN = Ammonium nitrate; UAN = Urea ammonium nitrate; UAS = Urea ammonium sulfate, Source: Doyle (2013)

2.6.3 Nitrogen application rate

Application of adequate N rate is very important for efficient use of N fertiliser and to maintain the economic sustainability of the cropping systems. Over-application of N fertilisers is economically not favourable because incremental increases in yield decreases with increasing amounts of N applied (Miner and Smith 1983) and it could result to detrimental effects on the quality of soil and water resources (Mackown et al. 1999). Long-term research on N fertility has shown that residual soil NO_3-N increases when N fertilization rates exceeded that required for maximum yield (Halvorson and Reule 1994; Westerman et al. 1994; Raun and Johnson 1995; Porter et al. 1996). Increased levels of NO_3-N in the soil profile maximize the potential of leaching NO_3-N below the root zone into shallow water zones, creating environmental pollution (Halvorson et al. 2001). Dinnes et al. (2002) reported that fertiliser N management, especially rate and timing of application, plays a dominant role on losses of NO_3-N from crop root zones.

In relation to liquid foliar application rates and leaf scorch, Abad et al. (2003) incorporated two foliar urea sprays applied at 50 kg N ha^{-1} (flag leaf stage) in N fertilisation studies conducted in the Mediterranean conditions of Spain. The authors reported minor leaf tip burn but plants recovered their green colour within a few days after N application. Subedi et al. (2007) added liquid foliar N topdressing treatments using urea solution applied at 40 kg N ha^{-1} (at boot stage) in studies conducted in Canada. Although no added benefit in terms of wheat grain yield was observed from additional foliar N topdressing, the authors did not report any major problems with leaf burn. In a recent study, Walsh and Christaens (2016) tested N source and dilution ratios (100/0, 33/66, 66/33) at N rate of 45 kg ha^{-1} (at early tillering) on NUE, grain yield and grain protein content amongst other parameters in studies conducted in the USA. These researchers reported that, although leaf burn was visible following application of liquid N fertilisers, wheat plants recovered within the next 2-3 weeks after application. The physical damage did not result to any grain yield or quality penalties. The above scenarios show that it is possible to apply liquid foliar N sprays at amounts ranging between 30 and 60 kg N ha^{-1} at different growth stages of the wheat plant and the challenges associated with leaf burn seem to be site specific depending on the environmental conditions during the time of application.

2.6.4 Nitrogen application time

The NUE of crops is influenced by rate and time of N fertiliser application (Ellen and Spiertz 1980; Fageria and Baligar 1999). Split applications of N to sandy soil and in high rainfall areas are common practice. Split applications of fertiliser N improved NUE for wheat genotypes when compared to pre-plant N applications (Olson and Swallow 1984). Wuest and Cassman (1992) similarly reported that N supplied late in the season could increase grain protein and NUE of wheat compared to pre-plant N application. Research done by Fageria and Baligar (1999) showed that agronomic efficiency of N for lowland rice was higher when N was applied in three split applications (one-third at sowing + one-third at active tillering + one-third at panicle initiation) compared to all N applied at sowing. Gholami et al. (2011) reported that foliar urea application time had significant effects on grain mass, number of seeds per spike, plant height, and protein content. According to these authors, urea applied at grain filling stage demonstrated a significantly greater yield compared to other stages of application. Combined foliar application of the three major nutrients (NPK) at the rate of 1% each in two equal splits at 30 and 60 days after planting (DAP) increased maize productivity under moisture stress conditions compared to one application of these nutrient elements (Amanullah et al. 2014). An earlier study by Amanullah et al. (2013) revealed that plant height, leaf area, biomass and grain yield was increased when foliar-N was applied late (45 and 60 days after emergence (DAE) compared to early (15 and 30 DAE) application in maize.

2.7 Nitrogen use efficiency (NUE) and its components

The NUE is expressed as the maximum yield output per unit amount of N applied, absorbed, or utilized by the crop for the production of grain and straw (Cassman et al. 2002; Ladha et al. 2005). Literature offers a number of different definitions, but most of them highlight the ability of a system to convert inputs into outputs. Agronomic efficiency, physiological efficiency, agrophysiological efficiency, apparent recovery efficiency and utilization efficiency are the key categories through which NUE is studied (Fageria and Baligar 2001, 2003; Santos et al. 2003; Ladha et al. 2005). Evaluation of NUE in plants is a critical and useful method to determine the efficiency of applied fertilisers including their role in the improvement of crop yields. Nitrogen use efficiency of wheat includes 1. Agronomy (which monitors grain production per unit of N applied), 2. Environment (which monitors the contamination of ground water, eutrophication of surface waters and ozone depletion by release of N_2O) and 3. Economics (improvement of farmer's income) (Raun and Johnson 1999).

Amongst others, partial factor productivity (PFP_N) and nitrogen harvest index (NHI) are some of the commonly used NUE indices. Partial factor producitivity is a useful index because it does not require measurements of grain yield in unfertilized plots and grain and/or plant N uptake (Ladha et al. 2005). Partial factor productivity is usually used for making general comparisons across agronomic practices. Higher PFP_N indicates a higher amount of N supply, while lower values show productivity limiting deficit. Typical values of PFP_N range between 40-80 kg kg^{-1}. The rates higher than 60 kg kg^{-1} are common in very efficiently managed systems, at low N rates or low soil N supply (Panayotova and Kostadinova 2016). Nitrogen harvest index (NHI) is defined as the ratio between nitrogen uptrake in grain and N uptake in grain plus straw or shoot (Fageria 2014). The NHI is an important index in that it measures the retranslocation efficiency of absorbed N from vegetative plant parts to the grain. The NHI further measures N partitioning in crop plants which gives an indication of how efficiently the plant utilize acquired N for grain production (Fageria and Baligar 2003). High NHI is associated with efficient utilization of applied N (Fawcett and Frey 1983) and high protein yield (Welch and Yound 1980).

Some of the NUE components commonly studied are evaluated by applying the following formulas (Baligar et al. 2001; Fageria and Baligar 2003; Ladha et al. 2005):

2.7.1 Agronomic efficiency (AE)

Agronomic efficiency (AE) = $G_f - G_u/N_a$ = kg.kg^{-1} (1)

Where G_f is the grain yield of the fertilized plot (kg), G_u is the grain yield in the unfertilized plot (kg), and N_a is the quantity of nutrient applied (kg).

The nitrogen agronomic efficiency (NAE) evaluates the overall efficiency of the production system and is associated with the apparent recovery and physiological efficiency (Craswell and Godwin 1984). The challenge with this method is that the contributions from mineralization of organic soil N is overlooked based on the assumption that N fertilisation did not have additional positive or negative influence on plant uptake of soil N (Stevens et al. 2005). The authors, however, concluded that this difference method remains the most significant approach for fertiliser N experiments in soils that are fairly new where variability among plots in soil mineral N availability is still minimal.

2.7.2 Physiological efficiency (PE)

Physiological efficiency (PE) = ($Y_f - Y_u/N_f$-N_u) = kg.kg^{-1} (2)

Where Y_f is the total biological yield (grain + straw) of the fertilised plot (kg), Y_u is the total biological yield in the unfertilised plot (kg), N_f is the nutrient accumulation in the fertilised plot in grain and straw (kg), and N_u is the nutrient accumulation in the unfertilised plot in grain and straw (kg).

Physiological efficiency measures the ability of a crop to utilize N in grain yield synthesis and is influenced by environmental stresses and the plant genotype. According to Cassman et al. (2002), it has been argued that two factors that govern PE are, 1. mode of photosynthesis (C_3 or C_4 photosynthetic pathway) and 2. grain-N concentration. Ladha et al. (2005) suggested that grain-N concentration and mobilization efficiency are the major factors determining PE.

2.7.3 Agrophysiological efficiency (APE)

Agrophysiological efficiency (APE) = ($G_f - G_u$/ N_f-N_u) = kg.kg^{-1} (3)

Where G_f is the grain yield in the fertilised plot (kg), G_n is the grain yield in the unfertilised plot (kg), N_f is the nutrient accumulation by straw and grain in the fertilised plot (kg) and N_u is the nutrient accumulation by straw and grains in the unfertilised plot (kg).

2.7.4 Apparent nutrient recovery efficiency (ANR)

$$\text{Apparent nutrient recovery efficiency (ANR)} = (N_f - N_u/N_a) \times 100 = \% \qquad (4)$$

Where N_f is the nutrient accumulation by the total biological yield (straw + grain) in the fertilised plot (kg), N_u is the nutrient accumulation by the total biological yield in the unfertilised plot (kg), and N_a is the quantity of nutrient applied (kg).

The ANR provides an estimation of the efficiency with which the crop uses applied N fertiliser and is affected by climatic conditions that in turn influence both soil mineral N availability and crop growth. According to Legg and Meisinger (1982), N recovery under field conditions ranges between 50 and 60%, but it is possible to achieve higher efficiency (70 and 80%) if the application and timing is improved. Van Rensburg (1996) reported an ANR of 64% with a South African wheat cultivar under optimum irrigation management. Otto and du Preez (2010) found ANR values ranging from 34.56% with cultivar SST 876 to 39.22% with cultivar Steenbras under irrigation in their study on South African cultivars under different N management strategies where supplementary irrigation was used.

2.8 Physiological aspects of grain yield

The life cycle of cereal crops follows a phasic pattern with vegetative and reproductive stage as the two major phases (Farooq et al. 2014). Grain development occurs during the reproductive phase, which starts with the transformation of a vegetative meristem into an inflorescence and a floral primordium, ending with the grains attaining the maximum dry matter (physiological maturity). According to GRDC (2005), the entire reproductive phase is a sequential process and may be divided into subphases such as flowering initiation and development, development of male and female gametophytes, pollination and fertilization, and grain development. Yield potential of cereal grains is largely dependent upon either fertile florets formed before anthesis (Wang et al. 1996), which may differ due to genotypic difference (Miralles et al. 1998) or environmental effects (Serrago et al. 2008). Fischer (1985) suggested that the period closer to anthesis (from terminal spikelet initiation to anthesis) is of significant importance in determing the number of fertile florets at anthesis and final grain yield.

Abiotic stresses have a strong influence on the flowering initiation and development. For example, Boyer and Westgate (2004) noted that abortive ovaries and infertile pollens were very common effects of drought in maize. In addition to drought, temperature extremes also affected floral development. Nitrogen is one of the nutrient elements that influence the floret development from the third floret primordium onwards (Ferrante et al. 2013). Similarly, plant growth regulators also influence the floret development in cereals (Guo et al. 1995; Wang et al. 1999). For example, Wang et al. (1999) found that exogenous application of cytokinin (zeatin) promoted the floret development while exogenous application of abscisic acid, indole acetic acid and gibberellic acid inhibited the floret development.

In cereals, N limits grain and quality via effects on plant biomass and consequently on grain number, size, and protein concentration (Angus et al. 1993; Demotes-Mainard and Jeuffroy 2001). In terms of N management, early season N application results in the accumulation of dry matter by enhancing tiller number and large photosynthetic surface area (Morgan 1988). On the other hand, late application of N at or after the emergence of flag leaf does not increase the leaf area but increase N contents of vegetative parts and prolongation of leaf area is the major cause of the increase in yield (Spiertz and Van de Haar 1978; Pearman et al. 1979). Millard and Catt (1988) and Shiraiwa and Sinclair (1993) noted a strong relationship between N concentration and single leaf photosynthetic rates, which appeared to be associated with large fractions of leaf N composed in photosysnthetic enzymes. Consequently, plants deficient in N have lower photosynthetic rates, accumulate less dry matter and produce lower yields (Delden 2001; Zhao et al. 2009). The highlighted physiological responses suggest that N is a major component of a successful plant life cycle including the improvement of grain yield.

Nitrogen management strategies should therefore be developed in such a way that they have a limited negative effect on both vegetative and reproductive phases of the crop phenology.

2.9 Conclusions

Wheat is one of the most important and valuable food crops for all humans. Sustainable production of this crop is necessary as the world population is expected to reach around 9-10 billion by 2050. Nitrogen is one of the nutrient elements that often limit the production of grain crops including wheat. Research has shown that there may be an improvement of nitrogen use efficiency in cereals through the application of liquid nitrogen in supplementation to basal granular fertilizer sources. There is an indication that liquid N topdressings are less subject to surface runoff, microbial immobilization, volatilization and denitrification compared to granular soil applied N. One of the challenges regarding the use of liquid N topdressings is leaf scorch when applied as foliar sprays. However, several studies have shown that plants are able to recover within 2-3 weeks after application where injuries are noticed. Other studies have indicated that no yield penalties were found because of leaf scorch following liquid N spray application.

Under conditions where liquid N fertilisers are applied as foliar sprays, the uptake mechanisms are affected by the concentration of the spray solution, molecular weight, solubility, electric charge and the solution pH, in addition to the prevailing environmental (temperature, rainfall, etc.) at or just after the time of application. In terms of N management, the source of N, method of application, N application rate and the time of application play a key role in determining the responses of plants to the applied N. All these factors in turn will have a direct influence on the nitrogen use efficiency of the crop or system. Nitrogen use efficiency is expressed as the maximum yield output per unit amount of N applied, absorbed or utilized by the crop for the production of grain or straw. There is a number of different definitions available in literature but most of them highlight the ability of a system to convert inputs into outputs. Agronomic efficiency, physiological efficiency, agro-physiological efficiency, utilization efficiency, apparent recovery efficiency and nitrogen harvest index are some of the commonly used terms in expressing nitrogen use efficiency.

Physiologically, N limits grain yield and grain quality via effects on plant biomass and grain number, size and protein concentration. Early N application results in the accumulation of dry matter by enhancing tiller number and large photosynthetic surface area. Application of N at or after the flag leaf emergence increase N concentration of the vegetative parts and thus prolong the leaf area, which result in increase in yield. Plants deficient in N will have lower photosynthetic rates, accumulate less dry matter and produce lower yields. These physiological responses suggest that N is a major component of a successful plant life cycle including the improvement of grain yield. At farm level, N management strategies should encourage improved NUE; limit any negative effect on vegetative and reproductive phases of the crop, thereby improving both grain yield and quality.

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Chapter 3

Description of the methodology, localities and climatic conditions

3.1 Introduction

Farmers in the wheat producing areas of the Western Cape face many challenges including high nitrogen fertiliser costs. According to the ARC (2016), fertiliser costs contribute approximately 30% or more to the total production inputs per year in the Western Cape and nitrogen accounts for a significant portion of this 30%. These challenges require the introduction of new principles and practices that will potentially reduce fertilizer input cost per unit area while improving or maintaining grain yield and quality of wheat. This study seeks an alternative method of nitrogen top-dressing to the spring wheat crop during the season by comparing methods of application as well as N application rates. This chapter will describe the approach followed in the study, trial localities, climatic conditions and experimental procedures.

3.2 Field experiments

Two similar field experiments were conducted in the two traditional wheat-producing areas of the Western Cape Province, South Africa; namely, southern Cape (Caledon) and Swartland (Moorreesburg). Roodebloem [34º 13’31.55”S; 19º 26’13.76”E; 117 m above sea level (masl)] and Langgewens (33° 16' 33.96" S; 18° 42' 14.4" E; 91 masl) Experimental Farms located near Caledon and Moorreesburg respectively were identified as the two study sites (Figure 3.1). The experiments rotated from one site to another within the same locality yearly from 2013 to 2015 in both localities. The geographic location of the experiments is shown in Table 3.1. The two localities were chosen to represent different production potentials, climatic conditions and soils found in each sub-region. Standard conventional wheat production practices were followed for soil preparation, planting, pest and disease control and harvesting. Wheat crops were planted using the recommended sowing density of the selected cultivar following the recommended agronomic practices regarding fertilisation of phosphorus, potassium and micro-elements.

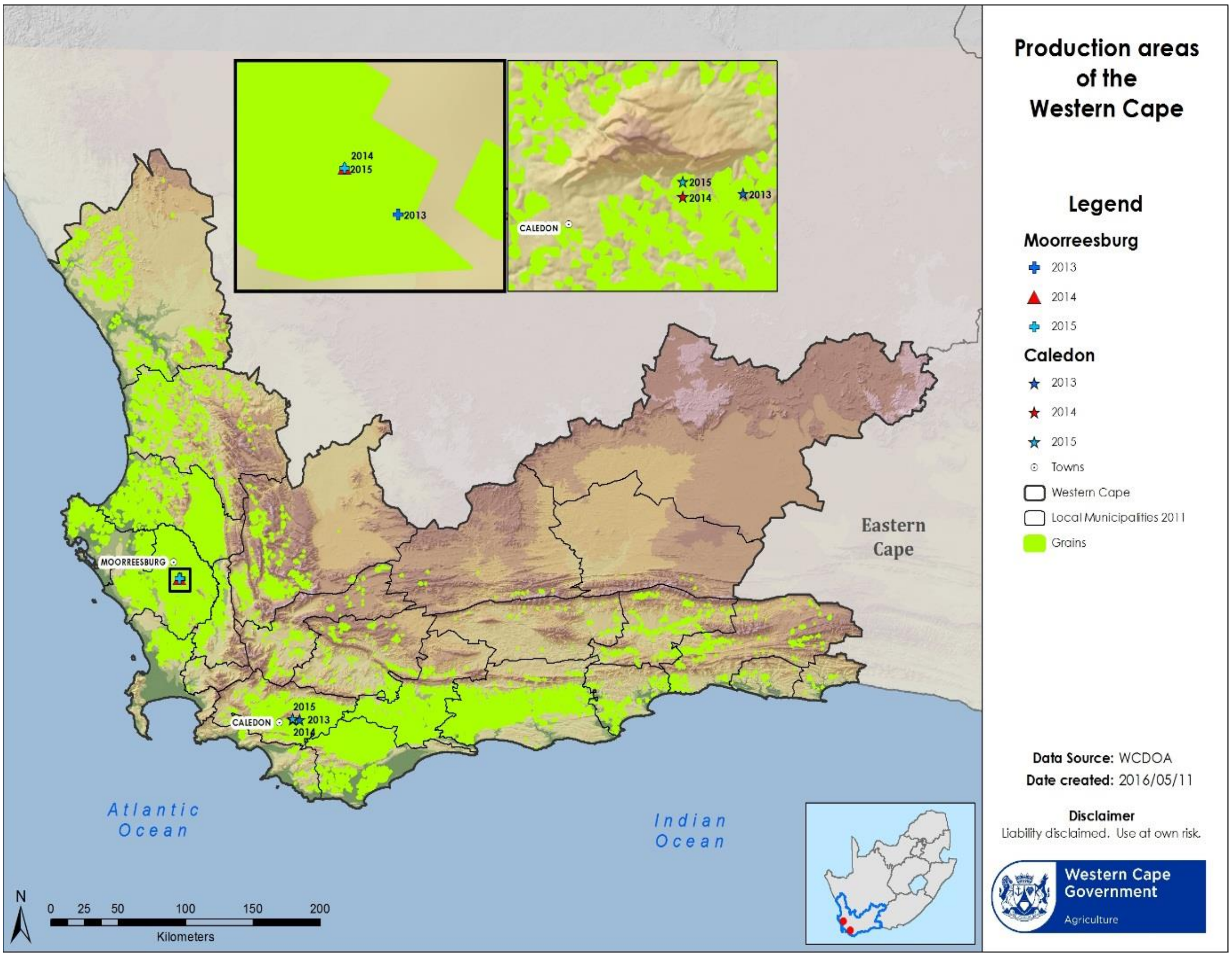

Figure 3.1 Map of localities and specific location of the field experiments within each locality from 2013-2015. (Source: Western Cape Department of Agriculture (WCDoA).

The experiments conducted at the Langgewens Experimental Farm were affected by herbicide resistant ryegrass populations in 2014. To reduce the effect of this resistant ryegrass in the following year, mechanical weed control methods were adopted and weeds were physically removed by hand hoe within the trial plots.

Table 3.1: Localities, farm name, latitude, longitude and previous crops during the study period 2013-2015

Locality	Farm name	Latitude			Longitude			Previous crop		
		2013	2014	2015	2013	2014	2015	2013	2014	2015
Caledon	Roodebloem	34.22843	34.22974	34.21987	19.5816	19.53073	19.53063	Oats	Canola	Oats
Moorreesburg	Langgewens	33.27521	33.27386	33.27521	18.27521	18.70931	18.70931	Medics	Medics	Oats

Table 3.2: General description of soils at Caledon (Roodebloem) and Moorreesburg (Langgewens) (Extracted fromTolmay 2008)

Locality	A-horizon	Sub-soil horizon	Estimated WHC*	Soil identification**
Caledon	0-30 cm Sandy loam (30% stone)	30-60 cm Sandy clay loam 70-80% hard shale (lithocutanic B-hor.)	Low - moderate	SaLm Gs 2211 non-red B
Moorreesburg	0-35 cm Sandy loam (30% stone)	35-50 cm Sandy clay loam 70-80% hard shale (lithocutanic B-hor.)	Low - moderate	SaLm Gs 2211 non-red B

*Estimated Water Holding Capacity

** Soil Classification Working Group

In 2015, the seasonal rainfall was below the long-term seasonal average and this affected the rainfed crop yields of the Western Cape but the impact was more severe in the Swartland compared to the southern Cape. (Table 3.4). In 2015, the monthly rainfall amounts were below long-term (2002-2012) monthly average rainfall for the majority of the months (Table 3.4).

3.3 Description of the soils

Field experiments were conducted at two localities in the Western Cape Small Grain Production areas; namely, Swartland (Moorreesburg) and southern Cape (Caledon) also known as the Rûens as indicated above. As previously mentioned, these two localities differ in terms of soil characteristics although there may be some similarities in some of the soil parameters depending on the exact location within the sub-region as well crop and cultivation histories of the specific sites involved.

3.3.1 Caledon (Roodebloem)

Caledon is generally known as a production area with high potential due to a fairly stable and high rainfall (Tolmay 2008). The soils found in this region are classified as Glenrosa 2211 with a non-red B-horizon (Soil Classification Working Group 1991) (Table 3.2). The A-horizon consist of a sandy loam texture and is about 30 cm deep with a stone and gravel fraction of 30%. The soils are generally associated with a low to moderate water holding capacity. The lithocutanic B-Horizon is 60 cm deep and has a low water holding capacity. The three (3) year soil fertility data is shown in Table 3.3. The soil carbon (C) content ranged between a low value of 0.86% and a high value of 3.35%. These variations were according to the year, specific site within this sub-region and the sampling depth as can be seen in Table 3.3 below. The variations in the C content of these soils can be associated with the history of cultivation in the experimental sites in which these studies were undertaken. The pH at this locality ranged between $pH_{(KCl)}$ 4.6 and 5.6 over the study period (Table 3.3) and as indicated with the C content; the differences were between specific locations of the experiment, year of sampling and the sampling depth. The soil phosphorus (P) (citric acid) levels were relatively high >30 mg kg^{-1} (DAFF, 2010) in all the sites, years and sampling depths. The lowest P value was 34 mg kg^{-1} at a depth of 15-30 cm in 2015 and the highest was 71 mg kg^{-1} at 0-15 cm in 2013. In terms of potassium (K), the lowest K value was 180 mg kg^{-1} and was obtained at a depth of 15-30 cm in 2014 while the highest value was 330 mg kg^{-1} obtained from 0-15 cm in 2013 as shown in Table 3.3. In terms of N analysis, Table 3.3 shows that the N percentage of the soil was relatively high in this locality. The lowest N levels were 0.17% and was obtained in 2013 and 2014 for 15-30 cm and 0-15 cm sampling depths respectively. The specific location used in 2015 proved to have the highest N levels in terms of N as a high value of 0.33% was observed in the topsoil followed by 0.28% of the subsoil in the same sampling year (Table 3.3).

3.3.2 Moorreesburg (Langgewens)

Within the Swartland sub-region, Moorreesburg represents a fairly large area. Generally, the topography is sloped and soils are shallow, containing a fairly high stone and gravel fraction (Tolmay 2008). The soil is classified as a Glenrosa 2211 containing a red B-horizon (Soil Classification Working Group 1991) as shown in Table 3.2. The A-horizon is about 35 cm deep with sandy loam texture and 30% stone and gravel fraction as shown in Table 3.2. The water holding capacity is low to moderate. The red-lithocutanic B-horizon is made up of 70-80% hard shale with low water holding capacity. The soil analyses for the three years of the study indicate that the soil C content was relatively low compared to the Caledon site and the values ranged between 0.4 and 0.87% (Table 3.3). A similar observation was reported by Tolmay (2008) where the Caledon site showed a C content of 2.8% compared to 1.4% found in Moorreesburg. As mentioned above with the Caledon site, the observed differences in the soil C content was between the sites, years and the sampling depth. The pH (KCl) in this locality ranged between 4.8 and 6.4 as shown in Table 3.3. The soil P levels were relatively high (>30 mg kg^{-1}) and the range was between 39 mg kg^{-1} and 133 mg kg^{-1}. It is clear that the specific site, year of sampling and the sampling depth contributed to the differences observed in Table

3.3 in terms of soil P levels. The K content of the soil in this locality was general lower than those observed in the Caledon site. The K levels ranged from low (<60 $mg.kg^{-1}$) to high (>120 $mg\ kg^{-1}$) (DAFF 2010) in this locality between the years 2013 and 2015.

Compared to Caledon, the N analyses showed that the Moorreesburg soils were low in N. The N levels ranged between 0.05% and 0.11% as influenced by the specific location of the experiment, the year of study and the sampling depth. The data shows however that there were very slight differences between the topsoil and the subsoil in terms of N in this locality. Table 3.1 shows that the experiments were conducted following the cultivation of medics, which were legumes at Langgewens in 2013 and 2014 compared to oats and canola for the same period at Roodebloem. However, the soil N levels in terms of N analysis favoured Roodebloem, which suggests that Caledon soils were generally better im terms of n content than soils found in Moorreesburg irrespective of the previous crop cultivated.

Table 3.3: Soil chemical properties in the topsoil (0-15 cm) and subsoil (15-30 cm) at the two localities from 2013-2015

Locality	Year	Soil depth	C	N	pH	P	Ca	Mg	K	Na
			(%)		(KCl)	mg kg^{-1}	cmol$^+$ kg^{-1}		mg kg^{-1}	
Roodebloem	**2013**	**0-15**	2.5	0.24	4.6	71	5.5	1.39	330	60
		15-30	1.68	0.17	4.6	47	3.52	0.94	182	62
	2014	**0-15**	1.83	0.17	5	68	5.04	1.1	201	58
		15-30	1.55	0.19	5.1	67	4.95	1.09	180	58
	2015	**0-15**	0.86	0.33	5.6	61	8.63	2.59	257	57
		15-30	3.35	0.28	5.5	34	6.94	2.34	242	53
Langgewens	**2013**	**0-15**	0.61	0.11	4.8	39	3.13	0.77	116	50
		15-30	0.87	0.09	5.1	39	3.37	0.82	59	53
	2014	**0-15**	0.57	0.06	5.8	133	3.33	1.25	248	15
		15-30	0.4	0.04	5.2	98	2.04	0.27	74	14
	2015	**0-15**	0.64	0.05	6.4	76	3.1	0.64	169	46
		15-30	0.52	0.05	6.2	76	2.95	0.65	122	30

3.4 Seasonal rainfall at the two localities

The rainfall data of the two localities in which this study was conducted is presented in a 10/11 day format (May – November) as shown in Table 3.4. The rainfall received between January and April was categorized as pre-season rainfall, while rainfall received in May to November months was recorded as in-season rainfall (planting to harvesting period). Planting commenced in May throughout the duration of the study and harvesting was conducted in October/November at both localities. For this reason, the rainfall received in the December month was excluded. The seasonal (pre- and in-season) rainfall is therefore represented by January to November months. According to the long-term (2002-2012) rainfall data of these localities, a comparison of seasonal rainfall (January to November) indicates that Roodebloem was generally better with a long-term mean of 502 mm compared to the mean of 362 mm at Langgewens (Table 3.4). The rainfall data from 2013-2015 shows that the rainfall patterns in terms of distribution favoured the Roodebloem site. Of these three years, Roodebloem received 83, 132, and 38 mm pre-season rainfall compared to 82, 88 and 36 mm recorded in Langgewens for 2013, 2014 and 2015 seasons respectively as shown in Table 3.4. For both these localities, it is evident that a reasonable amount of rainfall was received in 2013 compared to the long-term mean rainfall.

Table 3.4: Rainfall (mm) data for 2013 to 2015 in 10/11 day periods for the Roodebloem and Langgewens Experimental Farms along with long-term (LT 02-12) seasonal average rainfall (ARC-ISCW)

		Roodebloem				Langgewens			
Month	**Date**	**2013**	**2014**	**2015**	**LT 02-12**	**2013**	**2014**	**2015**	**LT 02-12**
Jan-Feb		**26**	**81**	**22**	**40**	**27**	**49**	**17**	**14**
Mar		**22**	**17**	**11**	**22**	**7**	**22**	**15**	**8**
Apr		**34**	**34**	**5**	**57**	**48**	**17**	**4**	**27**
May	01-10	17	7	1		1	18	1	
	11-20	1	7	8		0	4	11	
	21-31	15	9	11		34	17	9	
	Total	**33**	**23**	**20**	**49**	**36**	**39**	**20**	**57**
Jun	01-10	49	56	61		65	46	26	
	11-20	7	25	14		17	19	22	
	21-30	8	21	17		16	17	10	
	Total	**63**	**102**	**92**	**64**	**98**	**82**	**58**	**66**
Jul	01-20	4	25	1		2	18	1	
	11-20	24	6	39		41	18	26	
	21-31	10	10	58		20	24	25	
	Total	**37**	**41**	**97**	**64**	**63**	**60**	**52**	**53**
Aug	01-20	58	7	2		3	10	5	
	11-20	27	8	3		57	19	9	
	21-31	50	33	37		55	38	11	
	Total	**135**	**48**	**42**	**70**	**116**	**67**	**26**	**59**
Sep	01-10	6	2	6		5	1	3	
	11-20	34	11	1		48	10	1	
	21-30	3	18	31		8	2	6	
	Total	**43**	**31**	**39**	**31**	**62**	**13**	**11**	**27**
Oct	01-10	3	6	1		0	5	3	
	11-20	15	2	7		3	0	0	
	21-31	42	1	1		22	4	3	
	Total	**61**	**9**	**9**	**45**	**25**	**9**	**6**	**17**
Nov	01-10	1	13	4		1	8	10	
	11-20	100	26	21		23	7	7	
	21-30	0	0	1		0	6	0	
	Total	**101**	**39**	**26**	**55**	**25**	**21**	**17**	**23**
Pre-season		**88**	**132**	**38**	**119**	**82**	**88**	**36**	**49**
In-season		**473**	**223**	**325**	**376**	**362**	**281**	**190**	**303**
Grand Total		**556**	**355**	**363**	**495**	**444**	**369**	**226**	**352**

3.5 Temperatures of the two localities

3.5.1 Temperatures at Roodebloem

Table 3.5 shows the monthly temperatures for the duration of 2013-2015 along with the long-term (2002-2012) monthly average temperatures for Roodebloem. The temperature patterns followed those of the long-term monthly average temperatures in this locality. For both the maximum and minimum temperatures, there was no evidence of temperature extremes when compared to the long-term data. The highest monthly average maximum temperature recorded was 27.2°C in November month of 2015 while 26°C was the highest monthly average temperature recorded in terms of the long-term data.

Over the three-year study period, the lowest monthly maximum recorded was 16.1°C in July 2015 and this corresponded with the lowest monthly maximum average of 18.2°C of the same month for the long-term temperatures as shown in Table 3.5. In terms of the minimum temperatures, the lowest temperature during the period of this study was recorded in August 2013 while the lowest long-term minimum average was 5°C in the July month. The highest minimum temperature recorded between 2013 and 2015 was 13.9°C in November 2014 compared to the 12.5°C recorded as the highest minimum for the long-term temperature. From the table below, it is evident that the seasonal monthly temperatures during this study were in line with the long-term monthly averages recorded over the period of 2002-2012.

Table 3.5: Maximum and minimum monthly average temperatures for the period 2013-2015 along with the long-term (2002-2012) maximum and minimum monthly average temperatures for Roodebloem (ARC-ISCW)

Month	Tmax-13	Tmin-13	Tmax-14	Tmin-14	Tmax-15	Tmin-15	LTTmax	LTTmin
				°C				
Apr	23.7	11.2	25.4	13.2	25.0	12.7	**24.9**	**12.3**
May	21.7	9.6	21.3	10.6	22.6	11.9	**21.5**	**9.4**
Jun	17.2	7.0	17.7	7.8	18.3	8.5	**18.8**	**6.5**
Jul	17.4	7.8	16.7	6.7	16.1	7.6	**18.2**	**5.0**
Aug	16.4	6.6	18.7	8.9	18.6	9.4	**19.1**	**5.5**
Sep	17.6	6.9	20.4	8.7	21.4	9.6	**21.4**	**7.8**
Oct	21.3	10.6	24.7	12.1	25.7	12.6	**23.5**	**10.5**
Nov	25.0	13.4	25.7	13.9	27.2	13.0	**26.0**	**12.5**

Tmax = Maximum temperature; Tmin = Minimum temperature; LTmax = Long-term maximum temperature; LTmin = Long-term minimum temperature

3.5.2 Temperatures at Langgewens

From Table 3.6 below, it is shown that the average monthly temperatures during the three seasons of the study followed a similar pattern to that of the long-term monthly average temperatures for the majority of the months. There is however, a noticeable decrease in the month to month maximum temperatures in the three year period compared to the long-term mean monthly maximum temperatures for the majority of the months. In contrast, the mean minimum monthly temperatures during the study period tended to be higher than those observed from the long-term monthly averages. The highest maximum monthly average temperature recorded was 27.4°C in November 2014 while the lowest maximum was 16°C in July 2015. The long-term data also shows that November recorded the highest monthly average temperature (26.4°C) as shown in Table 3.6. The lowest maximum monthly average temperature is 18.2°C in August month for the long-term data, which does not correspond, with the month of July recorded in 2015. In terms of minimum temperatures, the lowest minimum monthly average was 6.9°C and was recorded in September month. From the long-term data, the lowest minimum temperature (5.6°C) was recorded for July. The highest of the minimum temperatures was recorded in January (14.6°C) compared to 11.3°C during April recorded for the long-term. Overall, the temperatures during the period of the study were not excessively high compared to the long-term temperatures.

Table 3.6: Maximum and minimum monthly average temperatures for the period 2013-2015 along with the long-term (2002-2012) maximum and minimum monthly average temperatures for Langgewens (ARC – ISCW)

Month	Tmax-13	Tmin-13	Tmax-14	Tmin-14	Tmax-15	Tmin-15	LTmax	LTmin
				°C				
Apr	24.1	11.8	27.4	14.6	25.9	13.5	**26.0**	**11.1**
May	18.7	9.5	20.6	10.7	21.6	10.7	**21.3**	**9.1**
Jun	16.5	8.3	17.1	8.3	17.3	8.6	**18.6**	**6.7**
Jul	17.3	8.1	16.8	7.7	16.0	7.1	**18.3**	**5.6**
Aug	16.2	7.3	17.9	9.1	17.9	8.6	**18.2**	**6.0**
Sep	17.4	6.9	20.7	8.5	21.7	9.2	**20.7**	**7.5**
Oct	23.4	10.0	26.7	12.4	26.5	11.6	**24.0**	**9.5**
Nov	27.0	13.3	27.4	13.3	26.8	12.5	**26.4**	**11.3**

Tmax = Maximum temperature; Tmin = Minimum temperature; LTmax = Long-term maximum temperature; LTmin = Long-term minimum temperature

3.6 Soil and grain quality analyses

For field experiments, soil samples were collected at depths of 0-150 mm and 15-300 mm just prior to planting to assess the general fertility status of the soil at pre-plant for each locality. To reduce the changes in the chemical composition of the soil after sampling, soil samples were transported in a cooler-box from the field to the laboratory and were immediately spread open in shallow pans, and dried in an oven with a fan at 60°C for 48 hours. Soil samples were thereafter sifted and stored at 10°C until analysis. The extractable P, Na, Ca and Mg were determined using citric acid (1%) method of analysis, extractable Cu, Mn and Zn was conducted using di-ammonium EDTA and extractable B by the hot water technique. The Walkley-Black method was used to determine the organic carbon content, while the soil pH was determined following the KCl extraction method (Non-Affiliated Soil Analysis Work Committee 1990). Nitrogen was determined following the Kjeldahl procedure. This analysis was performed at the soil testing laboratory of the Institute for Plant Production, Elsenburg (Western Cape Department of Agriculture, WCDoA).

Successive soil samples were collected at similar depths at tillering, just prior to the application of treatments to determine the N status of the soil. Six samples were collected randomly at a soil depth of 0-150 mm and 150-300 mm and the main aim of this sampling was to analyse the soil for total nitrogen. The collected samples were combined to a one composite sample representing the site because all treatments received 30 kg N ha^{-1} at sowing.

At flowering, two soil samples were collected randomly in each plot at 0-150 mm and 150-300 mm soil depths. In total, there was 33 soil samples at each soil depth (11 treatments x three replicates) and the three samples from the three replicates (per treatment) were combined to represent a treatment. This means that the soil total N analysis at flowering consisted of a combination of soil samples from three replicates (representing a treatment) mixed in one plastic bag for the top soil (0-150 mm) and the subsoil (150 – 300 mm). The total N content of the soil was determined using a Leco-combustion analyser (Leco FP-2000, Nitrogen/Protein Analyser; Leco Corporation, St Joseph, MI, USA) at BemLab [BemLab (PTY) Ltd, 16 Van der Berg Crescent, Gant's Centre, Strand, 7137].

Prior to combine harvesting, above ground biomass samples (1 m^2) of each treatment combination were collected at physiological maturity, dried until constant weight (60°C for 48 hours) and weighed. These samples were collected for the purpose of calculating plant biomass and harvest index as the combine harvester fails to accommodate these parameters at harvesting. The grain was separated from the residue and the residue was milled (<0.2 mm) and the total N (plant N) content determined with a Leco-combustion analyser (Leco FP-2000, Nitrogen/Protein Analyser; Leco Corporation, St Joseph, MI, USA). Grain protein was determined at the Welgevallen Experimental Farm at the University of Stellenbosch using the Near-Infrared Reflectance method for protein in wheat flour AACC Method 39-11 (American Association of Cereal Chemists 2000a). Crop yields were measured and grain samples were cleaned with air to remove foreign material. Following this, the hectolitre mass (kg hl^{-1}) and thousand kernel mass (TKM) were determined. Hectolitre mass was determined according to the AACC Method 55-10 (American Association of Cereal Chemists 2000b). The TKM was determined for each sample by counting 500 kernels with a Numigral seed counter and multiplying the mass by two.

3.7 Glasshouse experiments

The second component of this study consisted of two experiments that were conducted under controlled glasshouse conditions at the University of Stellenbosch, Stellenbosch, South Africa (33°56'33"S, 18°51'56"E, 136 m.a.s.l.). The purpose of conducting these glasshouse experiments was to substantiate information gaps that may arise from the field experiments resulting from external factors that may not be easily controlled under field conditions.

Two experiments were established under controlled glasshouse conditions in 2013-2016. One experiment (Experiment 1) was conducted in 2013, 2014 and 2016 while the second experiment (Experiment 2) was conducted in 2014, 2015 and 2016. The main aim of these experiments was similar to broader objectives of the field experiments as indicated in Chapter 1. The objective of the first experiment was to evaluate the effect of N topdressing method [granular (broadcast) and liquid (spray formulations)] and N rate on nitrogen use efficiency, grain yields and quality parameters of spring wheat. The N topdressing treatments were applied at tillering growth stage (Zadoks GS 21-29) (Zadoks et al. 1976). The second experiment was included to study the effect of N topdressing method, N rate and time of N topdressings [single versus split applications, once at tillering or twice at tillering and flowering (Zadoks GS 59)] on nitrogen use efficiency, grain yields and quality parameters of spring wheat.

The pots were filled with the topsoil (0-150 mm) obtained from the Welgevallen Experimental Farm as a growth medium. To achieve uniformity in the soil, the soil was gathered in heaps, mixed and ran through a 5 mm screen to separate the larger soil aggregates. A composite soil sample was collected and analysed to determine the soil chemical properties. Extractable P (Bray II), K, Na, Ca, Mg and S were determined using the citric acid (1%) method of analysis. The extractable Cu, Mn, and Zn by di-ammonium EDTA and the extractable B by the hot water technique were determined at BemLab. The Walkley-Black method was used to determine the organic carbon content following the Non-Affiliated Soil Analysis Work Committee (1990). The variation in soil properties between the years was ascribed to the fact that the soil was collected in different spots within the experimental farm.

Cultivar SST 027 seeds were sown in pots that was placed on top of steel tables in the glasshouse. Five seeds were sown per pot and seedlings were thinned to three plants per pot approximately 7 to 10 days after emergence. A nitrogen free nutrient solution was prepared and plants were watered manually every two to three days until physiological maturity. The amount of water applied was determined by adding water to the pot until the soil was saturated using a measuring cylinder. The amount of water added to achieve soil saturation in the first pot was used as a baseline to determine the amount of water to be applied in all other pots.

After harvesting, the above-ground biomass samples were collected, dried until constant weight (60°C for 48 hours), weighed and threshed. Number of ears (spikes), mass of ears, plant biomass and grain yield were determined per pot for each treatment. Plant N analysis, grain protein content and the falling number was then determined following the similar procedures stated above for field experiments. The samples from replicates of each treatment were combined to represent each treatment for all the quality related analyses. The treatments were combined because the mass of sample per replicate (glasshouse experiments) was too small to meet the minimum mass (g) of sample requirements for the standard procedures.

3.8 Description of experimental procedures

The experimental design was a Complete Randomised Block Design (CRBD) with 11 N topdressing treatments in the field studies, ten (10) treatments in glasshouse experiment 1 and 13 treatments in glasshouse experiment 2. The different treatment allocations of the different experiments are shown in Tables 3.7, 3.8, and 3.9. The same experimental procedures were followed for both localities in all the years.

The N topdressing rates ranged from 0 kg N ha^{-1} to 60 kg N ha^{-1} depending on the treatment. The total N applied per treatment varied between 0 and 90 kg N ha^{-1}. The N sources used were limestone ammonium nitrate (28%), urea ammonium nitrate (32%), and urea (46%) and these were applied as either solid (granular) or liquid (spray) at rates equivalent to 30 and 60 kg N ha^{-1}. An equivalent of 30 kg N ha^{-1} was applied at sowing to maintain plant growth between sowing and treatment application period. In the field experiments, the liquid N fertilisers were applied using a CP3 Knapsack (back-pack) sprayer. For the glasshouse experiments, the liquid N topdressing treatments were applied using a specially designed pot-spraying apparatus obtained from the Department of Agronomy, University of Stellenbosch. Activate N; which is a commercially available concentrated freeze dried formulation of *Bacillus* spp. and *Herbaspirillum* spp. was applied as a spray in selected treatments (as shown in respective tables below). According to the manufacturer, Activate N is a plant growth promoting inoculant containing a combination of micro-organisms designed to enhance nutrient utilisation, especially N utilisation [Madumbi Sustainable Agriculture (Pty). Ltd., Postnet Suite 148, Private Bag X 9118, Pietermaritzburg, 3200, support@madumbi.co.za, www.madumbi.co.za].

Table 3.7: Nitrogen fertiliser treatments for field experiments

Treatment	Method	Sowing (LAN) (kg ha^{-1})	Tillering (kg ha^{-1})	Total N (kg ha^{-1})
0 N	0	30	0	30
LAN 30+	0	30	30 + Activate N	60
LAN 60+	0	30	60 + Activate N	90
LAN 30	Granular	30	30	60
LAN 60	Granular	30	60	90
Urea 30	Granular	30	30	60
Urea 60	Granular	30	30	90
Urea 30	Liquid	30	30	60
Urea 60	Liquid	30	60	90
UAN 30	Liquid	30	30	60
UAN 60	Liquid	30	60	90

LAN = Limestone Ammonium Nitrate (28%), UAN = Urea Ammonium Nitrate (32%), LAN 30/60+ = LAN was followed by a foliar application of Activate N

Table 3.8: Nitrogen topressing treatments in the glasshouse experiment 1 in 2013, 2014 and 2016

Treatment	Method	Sowing (LAN) (g pot^{-1})	Tillering
Control	0	0	0
LAN 30+*	0	0.32	0.32 g pot^{-1}
LAN 30	Granular	0.32	0.32 g pot^{-1}
LAN 60	Granular	0.32	0.64 g pot^{-1}
Urea 30	Granular	0.32	0.19 g pot^{-1}
Urea 60	Granular	0.32	0.39 g pot^{-1}
Urea 30	Liquid	0.32	81.5 g 500 ml^{-1}
Urea 60	Liquid	0.32	163 g 500 ml^{-1}
UAN 30	Liquid	0.32	88.75 ml 500 ml-1
UAN 60	Liquid	0.32	177 ml 500 ml^{-1}

*LAN applied at 30 kg N ha^{-1} followed by a spray application of Activate N. The treatment was substituted with UAN 90 kg N ha^{-1} topdressing in 2016.

Table 3.9: Nitrogen topdressing treatments in glasshouse experiment 2 in 2014, 2015 and 2016

Treatment	Method	Sowing (LAN) (g pot^{-1})	Tillering	Flowering
Control	0	0	0	0
Urea 30 Single	Granular	0.21	0.13 g pot^{-1}	0
Urea 30 Split	Granular	0.21	0.07 g pot^{-1}	0.07 g pot^{-1}
Urea 60 Single	Granular	0.21	0.26 g pot^{-1}	0
Urea 60 Split	Granular	0.21	0.13 g pot^{-1}	0.13 g pot^{-1}
Urea 30 Single	Liquid	0.21	81.5 g 500 ml^{-1}	0
Urea 30 Split	Liquid	0.21	40.75 g 500 ml^{-1}	40.75 g 500 ml^{-1}
Urea 60 Single	Liquid	0.21	163 g 500 ml^{-1}	0
Urea 60 Split	Liquid	0.21	81.5 g 500 ml^{-1}	81.5 g 500 ml^{-1}
UAN 30 Single	Liquid	0.21	88.75 ml 500 ml^{-1}	0
UAN 30 Split	Liquid	0.21	44.38 ml 500 ml^{-1}	44.38 ml 500 ml^{-1}
UAN 60 Single	Liquid	0.21	177.5 ml 500 ml^{-1}	0
UAN 60 Split	Liquid	0.21	88.75 ml 500 ml^{-1}	88.75 ml 500 ml^{-1}

The experimental plots were 1.36 m wide and 6 m long with 8 rows fitted per plot with a row spacing of 17 cm. The planting of the crop commenced in May for both localities with only a few days separating the two planting times. Table 3.10 shows that planting commenced earlier in Langgewens compared to Roodebloem in two of the three years of the study. However, in 2014, sowing commenced three days earlier in Roodebloem than in Langgewens. Prior to combine harvesting, plants were sampled manually in a 1 m^2 area in the middle of the plots in order to obtain biomass yield and grain yield as the plot-harvesting machine only catered for grain harvesting of the entire plot. At harvesting, the Hege 125 B Combine Harvester designed for plot harvesting was used and the whole plot was harvested. Both sampling and harvesting commenced when the crop was physiologically mature and harvest ready. The yield plot^{-1} was converted into kg ha^{-1} by conversion method. Grain harvested in the 1 m^2 sampling and the remaining grain harvested by combine harvester were combined to represent the total grain per plot. The general agronomic practices followed during the duration of this study are shown in Table 3.11.

Table 3.10: Sowing dates, harvesting dates and the growing period (number of days between sowing and harvesting) at each locality between 2013 and 2015

Locality	**Sowing dates**			**Harvest dates**			**Growing period (days)**		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Roodebloem	20-May	12-May	18-May	28-Nov	17-Nov	18-Nov	193	188	183
Langgewens	14-May	15-May	13-May	12-Nov	13-Nov	27-Oct	189	181	166

Table 3.11: Summary of agronomic management practices followed at the two localities of the study

Activity	**Method**
Pre-plant soil preparation	Tine till and disc
Sowing density	100 kg ha^{-1}
Sowing machine	Plotman planter
Nutrient application at sowing	30 kg N ha^{-1}, 20 kg P ha^{-1} and 25 kg K ha^{-1}
Weed control	Axial (Pinoxaden - 100 g L^{-1}, cloquintocet-methyl - 25 g L^{-1}) at Roodebloem, Axial and MCPA (Potassium salt, phenoxy compound - 400 g L^{-1}) at Langgewens
Fungal disease control	Opus (Epoxiconazole - 125 g L^{-1}) at both localities
Harvesting machine	Hege 125 B

3.9 Statistical analysis and presentation of the data

Data obtained from the studies were analysed with the aid of statistical software Statistica (Version 13.2) (StatSoft Inc. Tulsa, OK, USA). A one-way Analysis of variance (ANOVA) was performed for each parameter at each locality for each season. The Coefficient of variance (Cv) (%) and least significant difference (LSD $_{(0.05)}$) values were calculated using Statistica at the p=0.05 confidence level.

Since the experiments were not laid out as a factorial design, the analyses of the method of application (granular and liquid), N rate (30 and 60 kg ha^{-1}), timing of application (single and split) and interactions were performed by extracting the relevant data sets based on the intended purpose. In the field experiments, the effect of method of application and N rate were analysed by extracting data from treatment denoted granular and liquid (Table 3.7). This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rate levels; 30 and 60 kg N ha^{-1}) factorial. The effect of year and locality was analysed using the data from the two localities for a period of three years per locality and the year x locality interaction was analysed as a 3 (years) x 2 (localities) factorial design.

In the glasshouse experiment 1, a one way ANOVA was performed using Statistica for each parameter for each year. The Coefficient of variance (Cv) (%) and least significant difference ($LSD_{(0.05)}$) values were calculated using Statistica at the p=0.05 confidence level. The effect of the method of N application was analysed by extracting data from treatments denoted as granular and liquid (Table 3.8). This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rate levels; 30 and 60 kg N ha^{-1}) factorial.

For the second glasshouse experiment, a one way Analysis of Variance (ANOVA) was performed using Statistica for each year. The Coefficient of variance (Cv) (%) and least significant difference ($LSD_{(0.05)}$) values were calculated using Statistica at the p=0.05 confidence level. The effect of the method of N application was analysed by extracting data from treatments denoted as granular and liquid (Table 3.9). This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rates: 30 and 60 kg N ha^{-1}) x 2 [timing of application: tillering (T) and tillering and flowering (TF)] factorial.

3.10 Description of cultivar characteristics

This study was conducted using a single cultivar known as SST 027. According to Sensako (2013), this cultivar is widely adapted and performs well at all yield potentials. SST 027 is a medium-long growth period cultivar with a good kernel attachment. The cultivar is resistant to stripe and moderately susceptible to stem and leaf rust. It produces grain of excellent quality.

3.11 References

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Chapter 4

Effect of granular and liquid applied nitrogen fertiliser topdressings on grain yields and quality parameters of spring wheat (*Triticum aestivum* L.) in the Western Cape Province, South Africa

Abstract

A field experiment was conducted at Roodebloem and Langgewens Experimental Farms of the Western Cape province of South Africa for three seasons (2013-2015) under dryland conditions, to evaluate the effect of nitrogen (N) fertiliser topdressing using granular and liquid sources of N applied at 30 and 60 kg ha^{-1} on spring wheat (*Triticum aestivum* L.). The experiment was a complete randomised block design with 11 N treatments replicated three times. The N sources were limestone ammonium nitrate (LAN), granular urea, liquid urea and urea ammonium nitrate (UAN) applied at tillering growth stage. The effect of the method of application (granular or liquid N) and N rate was analysed by extracting data from the granular and liquid N designated treatments and analysing it as a 2x2 factorial. The results showed that the effect of N treatment was inconsistent on grain yield (GY), hectolitre mass (HLM), thousand kernel mass (TKM), plant biomass and harvest index (HI) and water use efficiency (WUE). Nitrogen fertiliser treatment significantly affected GY in 2013 at Langgewens and in 2014 at Roodebloem. The effect of N rate showed that a significantly higher mean GY was produced through the application of N at 60 kg ha^{-1} (3 920 kg.ha^{-1}) compared to 30 kg ha^{-1} (3 577 kg.ha^{-1}) at Langgewens in 2013. At Roodebloem, the N rate x method of application interaction showed that GY was significantly improved when N was applied as a liquid at 30 kg ha^{-1} (3 318 kg ha^{-1}) compared to either granular N at 30 kg ha^{-1} (3 005 kg ha) or liquid N at 60 kg ha^{-1} (2 945 kg ha^{-1}). Large seasonal variations were observed both within the locality and between the two localities for tested parameters. The effect of locality showed that Roodebloem (3 090 kg.ha^{-1}) produced significantly higher mean GY compared to Langgewens (2 084 kg ha^{-1}). The locality x season interaction indicated that Langgewens produced the highest mean GY in 2013 probably due to favourable seasonal rainfall compared to other seasons and the other locality. The protein content and falling number were not affected significantly by N treatment. The multiple linear regression analysis showed that water use efficiency and to a lesser extent hectolitre mass significantly influenced grain yield for the two localities.

Keywords: Activate N, granular applied N, limestone ammonium nitrate (LAN), liquid applied N, Urea ammonium nitrate (UAN)

4.1 Introduction

Nitrogen (N) is regarded as one of the most critical plant nutrients and it is required in large quantities for growth and development of plants (Maali 2003). It is also considered as the nutrient that most often limit the production of crops (Ladha et al. 2005). Nitrogen serves as an important component of protein and chlorophyll and its application normally result in increased yields (Otto et al. 2000; Vlassak and Agenbag 2000). However, nitrogen fertilisers are the most expensive input in agricultural settings (Herrera et al. 2016). In South Africa, fertiliser inputs contribute on average between 30 and 50% to variable production costs of grain and oilseed producers (Grain SA 2011; AAC 2016). Consequently, nitrogen fertilisers contribute significantly to these production costs. The ultimate goal of each farmer is that the target crop utilises the applied N with the maximum efficiency.

Balasubramanian et al. (2004) stated that improvement of nitrogen use efficiency (NUE) may be possible with foliar urea or ammonium nitrate solutions. Compared to soil applied N, foliar-applied N is less subject to surface run-off, microbial immobilization, volatilization, and denitrification prior to absorption by the plant (Gooding and Davies 1992). Studies by Kara (2010) showed that wheat grain yield, nitrogen use efficiency, nitrogen uptake efficiency and grain protein content were higher in the late-season foliar N application compared to the conventional N application in a two-year study in Turkey. Saleem et al. (2013) reported that a three year pooled wheat grain yield data indicated that half the amount of N applied as soil applications and two sprays of 2% urea significantly increased the grain yield and was on par with soil application of urea. However, the concentration and uptake of nitrogen increased in grain following foliar application compared to soil application of urea due to efficient mobilization of N associated with foliar fertilisation. Woolfolk et al. (2002) observed a significant linear increase in grain N in five out of six site-years for pre-flowering applications of urea ammonium nitrate (UAN) in winter wheat.

From the above, it is clear that there are some benefits associated with foliar N applications compared to the traditional N applications. However, other studies have shown that there can be either no effect or a negative effect on wheat grain yield following foliar application of N. For example, Dick et al. (2016) found that late-season N treatment significantly improved grain protein, but did not significantly increase or decrease grain yield. Similarly, Woolfolk et al. (2002) indicated that no consistent increases or decreases from foliar N applications were observed for grain yield, or straw N in winter wheat. Evidence from different studies suggest that use of post-emergence foliar applied N tends to improve grain protein more compared to grain yield although not in all cases. A study by Alkier et al. (1972) found that soil and foliar applied post-emergent N application improved protein content more than the equivalent amount of N applied as broadcast at seeding time in field studies during the 1971 season but not in 1972.

Dampney et al. (1995) found the largest grain protein response (+0.84%) to foliar N application was observed where control grain protein was low (<12.4%) and the lowest response (+0.2%) was observed where grain protein was high in the absence of foliar application. Dampney et al. (1995) further stated that grain protein content showed an almost linear response to additional foliar urea-N application rates of up to 100 kg ha^{-1} N, at both low (<12.2%) and high (>13.9%) base levels of grain protein. The authors highlighted that in practical situations applications are usually limited to around 40 kg ha^{-1} N because of the risk of leaf scorch, although applications of up to 60 kg ha^{-1} N are used successfully in commercial practice. Ransom et al. (2016) found that a post-anthesis foliar application of urea ammonium nitrate (UAN) applied at 33 kg ha^{-1} was effective in improving the grain protein content of hard red spring wheat.

In South Africa, reports on use of foliar applied N are still limited. Du Plessis and Agenbag (1994) reported that increasing foliar (liquid) N fertilisation increased N uptake and N concentration of the plant and resulted in a higher S uptake in wheat in a study conducted at Langgewens experimental farm near Moorreesburg. Increase in N fertilisation also improved the baking quality due to higher protein content of the grain and flour. The aim of the current study is to evaluate the effect of granular N (LAN and urea) and liquid N (urea solution and UAN) topdressings applied at two different rates (30 and 60 kg ha^{-1}) on wheat grain yield, grain quality parameters and water use efficiency.

4.2 Materials and methods

An experiment was conducted from 2013 to 2015 at Roodebloem (34°22´ S, 19°52´ E; 117 m above sea level (masl)) (near Caledon) and Langgewens (33°17´ S, 18°40´ E; 91 masl) (near Moorreesburg) experimental farms in the Western Cape province of South Africa. The aim was to evaluate the response of spring wheat to granular and liquid applied N topdressings. The design was a randomized complete block design with 12 treatments replicated three times. The treatments are shown in Table 4.1. The sources of N used in the study were limestone ammonium nitrate (LAN 28% N), urea (46% N) solution, urea (46% N) granules, and urea ammonium nitrate (UAN) (32%). The application of the treatments was conducted at tillering stage (Zadoks GS 21-26) at 0 kg N ha^{-1}, 30 kg N ha^{-1} and 60 kg N ha^{-1} (Zadoks et al. 1974). However, this exercise was conducted at late stem extension/early heading (Zadok's 45-50) in 2015 due to unfavourable climatic conditions for spraying. Wind was the main factor, which resulted in the cancellation of N topdressing on three different occasions. Soil chemical properties of the two localities are given in Table 4.2. Soil samples (six sub-samples that were combined for analyses) were collected randomly in the designated experimental area prior to sowing at 0-15 and 15-30 cm for analysis to represent the general pre-plant soil chemical properties. Before the application of fertiliser N topdressing treatments (at tillering), a second set of soil samples was collected at similar soil depths to assess the N status of the soil (Table 4.3). A third and final soil sampling was collected during the flowering period to further assess the N status of the soil and the results are shown in Table 4.4. The summary of rainfall conditions during the three seasons of this study is presented in Figures 4.1 and 4.2, and data on temperature is shown in Tables 4.5 and Table 4.6. The monthly climatic data (2013-2014) was obtained from the Institute of Soil, Climate and Water of the Agricultural Research Council – ARC-ITSC, while the long-term data (2002-2012) was obtained from the South African Weather Services (SAWS).

Nitrogen was applied as LAN (28% N) in all the plots at sowing at 30 kg N ha^{-1}. The control treatment did not receive any N at treatment application time. At mid-tillering, the N topdressing treatments were applied using LAN (28% N) and urea (46% N) broadcast by hand on the plot to represent the granular N topdressing treatments, while urea solution (urea dissolved in water) and UAN (32% N) was applied as a liquid spray on the plant surface. The application rates of the treatments were 30 kg N ha^{-1} and 60 kg N ha^{-1}. In two other treatments, LAN (28% N) was applied at both 30 kg N ha^{-1} and 60 kg N ha^{-1} and was immediately followed by a liquid spray application of Activate N solution. Activate N is a commercially available concentrated freeze dried formulation of *Bacillus* spp. and *Herbaspirillum* spp. and these treatments are denoted as LAN+ in text. According to the manufacturer, this plant growth promoting inoculant (PGPR) is a unique combination of micro-organisms that has been designed to enhance nutrient utilisation, especially N utilisation [Madumbi Sustainable Agriculture (Pty). Ltd., Postnet Suite 148, Private Bag X 9118, Pietermaritzburg, 3200, support@madumbi.co.za, www.madumbi.co.za]. The summary of treatments is presented in Table 4.4. The experimental plots were 1.36 m wide and 6 m long with a row spacing of 17 cm and 8 rows were fitted per plot. The spacing was 1 m between plots and 2.5 m between replicates. The planting of the crop commenced in May for both localities with only a few days separating planting at the two localities.

Appropriate weed and pest management practices were applied when necessary. However, a herbicide resistant ryegrass (*Lolium spp.)* population at Langgewens affected the experimental plots in 2014 season although a weed control spraying program was adhered to.

Table 4.1: Nitrogen fertiliser treatments for field experiments

Treatment	Method	Sowing (LAN) (kg ha^{-1})	Tillering (kg ha^{-1})	Total N (kg ha^{-1})
0 N	0	30	0	30
LAN 30+	0	30	30 + Activate N	60
LAN 60+	0	30	60 + Activate N	90
LAN 30	Granular	30	30	60
LAN 60	Granular	30	60	90
Urea 30	Granular	30	30	60
Urea 60	Granular	30	30	90
Urea 30	Liquid	30	30	60
Urea 60	Liquid	30	60	90
UAN 30	Liquid	30	30	60
UAN 60	Liquid	30	60	90

LAN = Limestone Ammonium Nitrate (28%), UAN = Urea Ammonium Nitrate (32%), LAN 30/60+ = LAN was followed by a foliar application of Activate N

Above ground biomass samples (1 m^2) of each treatment collected at physiological maturity was dried until constant weight (60°C for 48 hours), weighed and threshed. The grain was separated from the residue and the residue was milled (<0.2 mm) and the total N concentration determined with a Leco-combustion analyser (Leco FP-2000, Nitrogen/Protein Analyser; Leco Corporation, St Joseph, MI, USA). Grain protein was determined at the Welgevallen Experimental Farm at the University of Stellenbosch using the Near-Infrared Reflectance method for protein in wheat flour AACC Method 39-11 (American Association of Cereal Chemists 2000a). Crop yields were measured and grain samples were cleaned with air to remove foreign material. Following this, the hectolitre mass (kg hl^{-1}) and thousand kernel mass (TKM) were determined. Hectolitre mass was determined according to the AACC Method 55-10 (American Association of Cereal Chemists 2000b). The TKM was determined for each sample by counting 500 kernels with a Numigral seed counter and multiplying the mass by two.

Table 4.2: Soil chemical properties in the topsoil (0-15 cm) and subsoil (15-30 cm) immediately prior to sowing at the two localities from 2013-2015

Locality	Year	Soil depth	C	N	pH	P	Ca	Mg	K	Na
			(%)		(KCl)	mg kg^{-1}	cmol^{+} kg^{-1}		mg kg^{-1}	
Roodebloem	**2013**	**0-15**	2.5	0.24	4.6	71	5.5	1.39	330	60
		15-30	1.68	0.17	4.6	47	3.52	0.94	182	62
	2014	**0-15**	1.83	0.17	5	68	5.04	1.1	201	58
		15-30	1.55	0.19	5.1	67	4.95	1.09	180	58
	2015	**0-15**	0.86	0.33	5.6	61	8.63	2.59	257	57
		15-30	3.35	0.28	5.5	34	6.94	2.34	242	53
Langgewens	**2013**	**0-15**	0.61	0.11	4.8	39	3.13	0.77	116	50
		15-30	0.87	0.09	5.1	39	3.37	0.82	59	53
	2014	**0-15**	0.57	0.06	5.8	133	3.33	1.25	248	15
		15-30	0.4	0.04	5.2	98	2.04	0.27	74	14
	2015	**0-15**	0.64	0.05	6.4	76	3.1	0.64	169	46
		15-30	0.52	0.05	6.2	76	2.95	0.65	122	30

Table 4.3: Total nitrogen (%) in soil samples taken at tillering stage before the application of treatments

Roodebloem			
Soil depth	**2013**	**2014**	**2015**
	mg kg^{-1}		
0-15	0.23	0.25	0.30
15-30	0.22	0.26	0.30
Total	**0.45**	**0.51**	**0.60**
Langgewens			
Soil depth	**2013**	**2014**	**2015**
	mg kg^{-1}		
0-15	0.23	0.20	0.10
15-30	0.22	0.16	0.10
Total	**0.45**	**0.36**	**0.20**

The water use efficiency (WUE) which is grain yield per millimetre of rainfall received was calculated using the following equation (Perry and Hillman 1991).

WUE (kg seed mm water^{-1}) = Grain yield (kg ha^{-1}) / Water use (mm)

Water use was calculated as:

Water use (mm) = 51.1 + 0.75 (May to October rainfall)

This equation was used in the Western Australian conditions, which are comparable to the conditions in the Western Cape and all the trials in this study were planted in May. This equation was considered relevant to use in this study as was also confirmed by Ngezimana and Agenbag (2015).

Analysis of variance (ANOVA) was performed using the GLM (General Linear Model) Procedure of Statistica 13.2 (Statsoft Inc., Tulsa, OK, USA)) to test for differences between treatments for all parameters. Means were separated using the Fisher's protected least significant difference (LSD) test at $p = 0.05$. In cases where residuals were not normally distributed, the Kruskal-Wallis test was used as a non-parametric test to confirm the results of the ANOVA. In cases where Levene's test for homogeneity of variances indicated heterogeneous variances, the LSD test was replaced with the Games-Howell multiple comparison procedure.

The possible interactions between method of application (granular or liquid) and N rate was analysed by extracting data from treatments denoted granular and liquid in Table 4.1 above. This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rate levels; 30 and 60 kg N ha^{-1}) factorial design.

The effect of year and locality was analysed using the data from the two localities for a period of three years per locality and the year x locality interaction was analysed as a 3 (years) x 2 (localities) factorial design.

The effect of N treatment on grain protein content (GPC) and the falling number (FN) was analysed using data sets from all the treatments. Since the quality parameters were analysed using the combined grain (from the three replicates), the statistical analysis was performed using the years (three years – 2013-2015) as replicates.

Multiple regression analysis was performed for all the tested variables and grain yield was used as the dependant variable to assess which variable/s contributed significantly to the grain yield and to further evaluate the relationship between the different variables. Where there was severe multi-collinearity among the input variables, the best five input variables were selected using the best subsets procedure and variables were then reduced to the best three or two input variables.

Table 4.4: Results of soil total nitrogen (%) for samples taken during the flowering growth stage of the plants from 2013 to 2015

		Total N (%)					
		Roodebloem			Langgewens		
Treatment	**Soil depth (cm)**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**
0 N	0-15	0.19	0.20	0.30	0.10	0.10	0.06
	15-30	0.15	0.20	0.20	0.09	0.10	0.05
	Total	**0.34**	**0.40**	**0.50**	**0.19**	**0.20**	**0.11**
LAN+ 30	0-15	0.20	0.20	0.35	0.10	0.10	0.06
	15-30	0.18	0.21	0.29	0.10	0.10	0.06
	Total	**0.38**	**0.41**	**0.64**	**0.20**	**0.20**	**0.12**
LAN+ 60	0-15	0.20	0.30	0.30	0.09	0.10	0.07
	15-30	0.21	0.25	0.24	0.09	0.09	0.06
	Total	**0.41**	**0.55**	**0.54**	**0.18**	**0.19**	**0.13**
LAN 30	0-15	0.16	0.20	0.30	0.09	0.10	0.06
	15-30	0.14	0.20	0.35	0.09	0.10	0.06
	Total	**0.30**	**0.40**	**0.65**	**0.18**	**0.20**	**0.12**
LAN 60	0-15	0.16	0.30	0.40	0.11	0.09	0.06
	15-30	0.17	0.25	0.35	0.07	0.10	0.06
	Total	**0.33**	**0.55**	**0.75**	**0.18**	**0.19**	**0.12**
Urea 30 S	0-15	0.20	0.21	0.31	0.09	0.10	0.06
	15-30	0.20	0.21	0.26	0.08	0.10	0.06
	Total	**0.40**	**0.42**	**0.57**	**0.17**	**0.20**	**0.12**
Urea 60 S	0-15	0.20	0.20	0.30	0.09	0.09	0.06
	15-30	0.21	0.20	0.24	0.09	0.10	0.05
	Total	**0.41**	**0.40**	**0.54**	**0.18**	**0.19**	**0.11**
Urea 30 L	0-15	0.22	0.20	0.20	0.10	0.10	0.05
	15-30	0.21	0.20	0.20	0.10	0.10	0.06
	Total	**0.43**	**0.40**	**0.40**	**0.20**	**0.20**	**0.11**
Urea 60 L	0-15	0.20	0.20	0.34	0.09	0.10	0.05
	15-30	0.20	0.20	0.32	0.08	0.10	0.06
	Total	**0.40**	**0.40**	**0.66**	**0.17**	**0.20**	**0.11**
UAN 30	0-15	0.15	0.20	0.30	0.10	0.10	0.08
	15-30	0.14	0.20	0.25	0.09	0.09	0.07
	Total	**0.29**	**0.40**	**0.55**	**0.19**	**0.19**	**0.15**
UAN 60	0-15	0.24	0.15	0.30	0.09	0.10	0.07
	15-30	0.18	0.20	0.20	0.09	0.10	0.06
	Total	**0.42**	**0.35**	**0.50**	**0.18**	**0.20**	**0.13**

Table 4.5: Maximum and minimum monthly average temperatures for the period 2013-2015 along with the long-term (2002-2012) maximum and minimum monthly average temperatures for Roodebloem

Month	Tmax-13	Tmin-13	Tmax-14	Tmin-14	Tmax-15	Tmin-15	LTTmax	LTTmin
	°C							
Apr	23.69	11.2	25.44	13.22	24.98	12.73	**24.93**	**12.27**
May	21.73	9.57	21.33	10.62	22.64	11.88	**21.45**	**9.43**
Jun	17.21	6.96	17.71	7.80	18.26	8.47	**18.80**	**6.45**
Jul	17.44	7.75	16.65	6.74	16.07	7.59	**18.22**	**4.99**
Aug	16.37	6.60	18.72	8.93	18.60	9.36	**19.05**	**5.52**
Sep	17.62	6.86	20.36	8.67	21.36	9.63	**21.37**	**7.76**
Oct	21.27	10.63	24.65	12.14	25.74	12.55	**23.47**	**10.51**
Nov	24.95	13.42	25.72	13.90	27.15	12.96	**26.02**	**12.48**

- Tmax = Maximum temperature; Tmin = Minimum temperature; LTmax = Long-term maximum temperature; LTmin = Long-term minimum temperature

Table 4.6: Maximum and minimum monthly average temperatures for the period 2013-2015 along with the long-term (2002-2012) maximum and minimum monthly average temperatures for Langgewens

Month	Tmax-13	Tmin-13	Tmax-14	Tmin-14	Tmax-15	Tmin-15	LTmax	LTmin
	°C							
Apr	24.06	11.83	27.36	14.61	25.90	13.48	**26.01**	**11.14**
May	18.72	9.51	20.62	10.72	21.63	10.70	**21.29**	**9.08**
Jun	16.52	8.33	17.09	8.29	17.34	8.64	**18.55**	**6.65**
Jul	17.26	8.13	16.82	7.67	16.01	7.14	**18.31**	**5.56**
Aug	16.16	7.26	17.91	9.11	17.89	8.56	**18.22**	**5.97**
Sep	17.43	6.92	20.72	8.49	21.72	9.19	**20.65**	**7.45**
Oct	23.41	10.04	26.71	12.43	26.47	11.63	**24.04**	**9.47**
Nov	27.02	13.29	27.37	13.34	26.82	12.52	**26.44**	**11.29**

- Tmax = Maximum temperature; Tmin = Minimum temperature; LTmax = Long-term maximum temperature; LTmin = Long-term minimum temperature

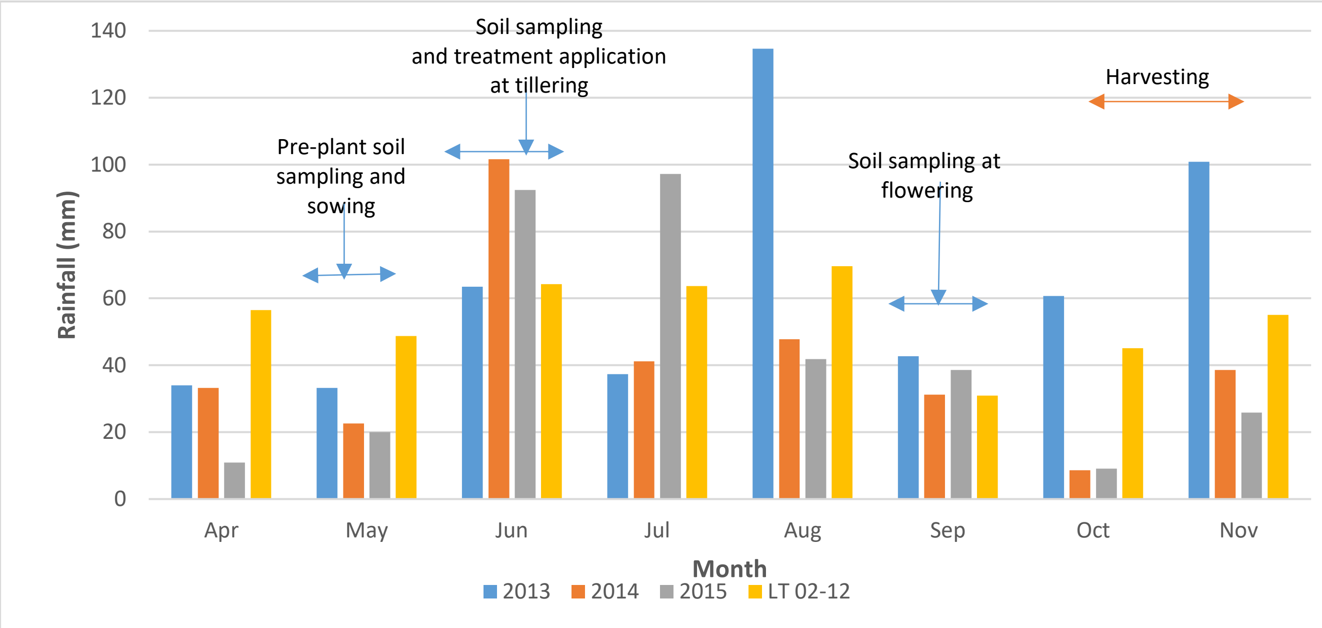

Figure 2.1: Monthly rainfall (mm) from 2013 – 2015 and long term (2002-2012) monthly average rainfall (mm) for the Roodebloem site

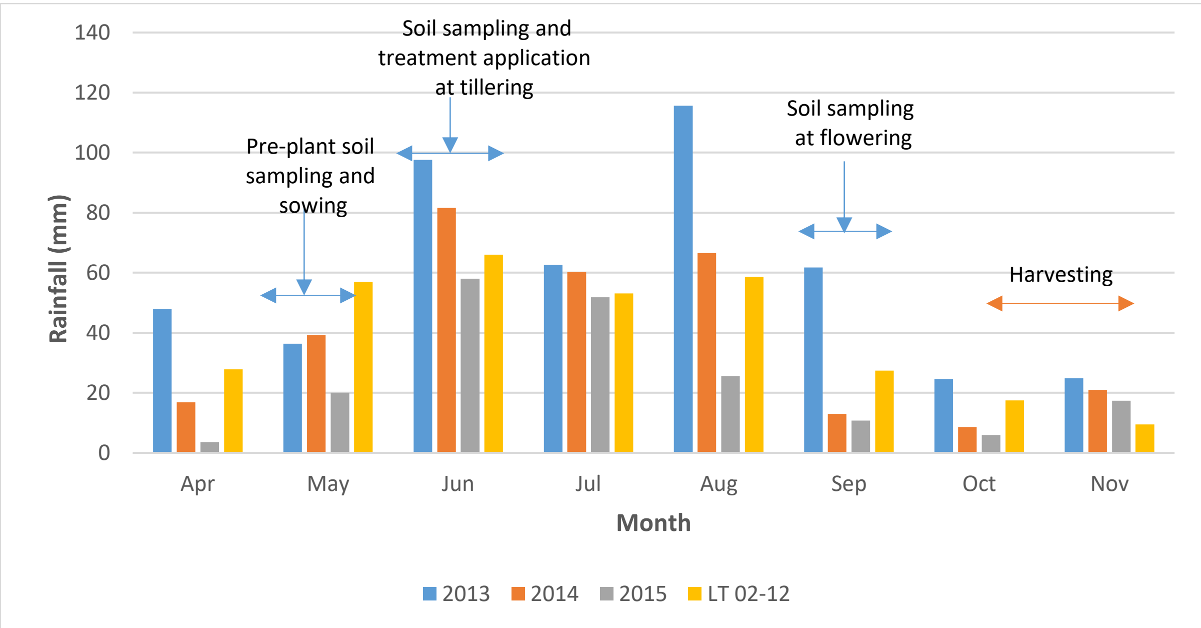

Figure 4.2: Monthly rainfall (mm) from 2013 – 2015 and long term monthly average rainfall (mm) (2002-2012) for the Langgewens site

4.3 Results and discussion

4.3.1 Significance of *p* values

The summary of Anova analysis is shown in Table 4.7. The N fertilizer topdressing treatment significantly ($p \leq 0.05$) affected grain yield (GY) at Roodebloem in 2014. The results showed that N fertiliser topdressing treatment had a significant effect on thousand kernel mass (TKM) and hectolitre mass (HLM) at Roodebloem in the 2013 but HLM was not significantly affected in 2014 (Table 4.7). Plant biomass (PB) and harvest index (HI) were significantly affected by the N treatment in the 2014 season at Roodebloem. At Langgewens, only the 2013 season showed a significant N fertiliser topdressing response on GY. A significant response resulting from N fertiliser treatment was observed for TKM at Langgewens in 2014. In 2015, the HLM was significantly affected by fertiliser N topdressing treatment at Langgewens.

Extracting selected data sets to investigate the effect of N rate and N application method showed that GY was significantly affected by the fertiliser N topdressing rate at Langgewens in 2013 (Table 4.8). The N topdressing fertiliser rate (30 kg N ha^{-1} or 60 kg N ha^{-1}) x method of application (granular or liquid N) interaction showed a significant effect on GY at Roodebloem in 2014.

The season (year) significantly ($p \leq 0.05$) affected the different studied parameters for both localities (Table 4.9). Similarly, there was a significant influence observed for the effect of locality, and the interaction between the season and locality for GY, TKM, HLM, PB, HI and water use efficiency (WUE). Due to the missing data for season one (2013) for PB and HI, the season, locality and season x locality interaction effect on these two parameters was not analysed.

The fertiliser N treatment, N rate, method of application and their interactions did not significantly influence the grain protein content (GPC) and the falling number (FN) (Data not shown). Data on the summary of the effect of N treatment on GPC and FN is shown in Table 4.13.

Table 4.7: Analysis of variance (ANOVA) results for grain yield (GY), thousand kernel mass (TKM), hectolitre mass (HLM), Harvest index (HI), plant biomass (PB), harvest index (HI) and water use efficiency (WUE) in response to different N treatments

Roodebloem	Source	GY	TKM	HLM	PB	HI
2013	N treatment	ns	*	*	-	-
2014	N treatment	*	*	ns	*	*
2015	N treatment	ns	ns	ns	ns	ns
Langgewens	**Source**	**GY**	**TKM**	**HLM**	**PB**	**HI**
2013	N treatment	*	ns	ns	-	-
2014	N treatment	ns	*	ns	*	ns
2015	N treatment	ns	ns	*	ns	ns

* Significant at the 0.05 probability level, ns = not significant at the 0.05 probability level. (- = Data missing)

Table 4.8: Analysis of variance (ANOVA) results for the effect of N rate, method of N application and the interaction between N rate and method of N application on grain yield (GY), thousand kernel mass (TKM), hectolitre mass (HLM), Harvest index (HI), plant biomass (PB) and harvest index (HI)

Roodebloem	Source	GY	TKM	HLM	PB	HI
2013	N rate	ns	ns	ns	-	-
	Method	ns	ns	ns	-	-
	N rate x Method	ns	ns	ns	-	-
2014	N rate	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns
	N rate x Method	*	ns	ns	ns	ns
2015	N rate	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns
	N rate x Method	ns	ns	ns	ns	ns
Langgewens	**Source**	**GY**	**TKM**	**HLM**	**PB**	**HI**
2013	N rate	*	ns	ns	-	-
	Method	ns	ns	ns	-	-
	N rate x Method	ns	ns	ns	-	-
2014	N rate	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns
	N rate x Method	ns	ns	ns	ns	ns
2015	N rate	ns	ns	ns	ns	ns
	Application method	ns	ns	ns	ns	ns
	N rate x Method	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level, ns = not significant at the 0.05 probability level, (- = Data missing)

Table 4.9: Effect of season, locality and the interaction between locality and season on grain yield (GY), thousand kernel mass (TKM), hectolitre mass (HLM), plant biomass (PB), harvest index (HI) and water use efficiency (WUE) for Roodebloem and Langgewens

Effect	GY	TKM	HLM	PB	HI	WUE
Season	*	*	*	*	*	*
Locality	*	*	*	*	*	*
Locality x Season	*	*	*	*	*	*

* Significant at the 0.05 probability level

4.3.2 Grain yield (GY)

At Roodebloem in 2013, the mean GY varied from 3 012 kg ha^{-1} in the urea solution treatment applied at 60 kg N ha^{-1} to 1 981 kg ha^{-1} from plants treated with granular urea at 60 kg N ha^{-1} but none of the treatments differed significantly from each other (*p≤0.05*) (Table 4.10). The lack of responses to applied N in 2013 could probably be a result of N losses through leaching or high N mineralization in the soil. The seasonal rainfall in 2013 exceeded the long-term seasonal rainfall by 14.39%. A four-year on-farm study by Schulz et al. (2015) found that different fertilisation treatments using calcium ammonium nitrate (CAN), urea and UAN were not significantly different either over the four years or in any of the four years. The authors suggested that the experimental soils (Luvisols) were characterized by high fertility and high mineralization potential, which enabled the plants to compensate short periods of N deficiency caused by insufficient fertiliser supply. Furthermore, Alcoz et al. (1993) and Maidl et al. (1996) reported that weather is the most important variable affecting grain yield followed by the total amount of applied N, application time and technique.

The GY responses differed significantly (*p≤0.05*) between treatments in 2014. Table 4.10 shows that the mean GY ranged between 3 388 kg ha^{-1} (UAN at 30 kg N ha^{-1}) and 2 818 kg ha^{-1} (60 kg N ha^{-1} liquid urea) in 2014. The poor responses showed by liquid urea at 60 kg N ha^{-1} could be due to urea phytotoxicity or volatilization of urea as NH_3 gas (Bremner 1995).

Although not significant, the effect of method of N application on GY tended to favour the liquid applied N compared to granular applied N in 2013 and 2014 seasons at Roodebloem

(Data not shown). Woolfolk et al. (2002) reported a significant linear increase in wheat grain yield in four out of six-site years for pre-flowering application of UAN, although these effects were not always consistent. This could be due to reduced N losses through leaching and denitrification associated with providing N through foliage compared to traditional soil application (Amanullah et al. 2015). According to Gooding and Davies (1992), foliar N applied before flag leaf emergence increases grain yield and this benefit can be even higher when N availability is limiting.

Extracting data sets to evaluate the effect of N rate x method of N application interaction on GY showed a significant response at Roodebloem in 2014 (Figure 4.3). The responses showed that there was a significantly higher mean GY (3 318 kg ha^{-1}) when N was applied as a liquid at 30 kg.ha^{-1} compared to granular N at 30 kg.ha^{-1} (3 005 kg ha^{-1}) or liquid N at 60 kg ha^{-1} (2 945 kg ha^{-1}). This was however, not significantly different to the mean GY obtained from the application of granular N topdressing treatment at 60 kg ha^{-1}. This could be an indication that application of N topdressing at relatively low levels (≤30 kg ha^{-1}) in a liquid form could be beneficial compared to liquid N application at higher rates. Kettlewell and Cooper (1994) reported that liquid foliar applied N at lowest rates increased grain yields in six out of nine winter wheat experiments in UK studies. Various other studies have also indicated that a small amount of N applied as liquid through foliage significantly increased grain yield of crops (Rauthan and Schinitzer 1981; Haq and Mallarino 2000). This could be due to the reduced timeframe between the application of the nutrient and the uptake by the plant (Ahmad and Jabeen 2005).

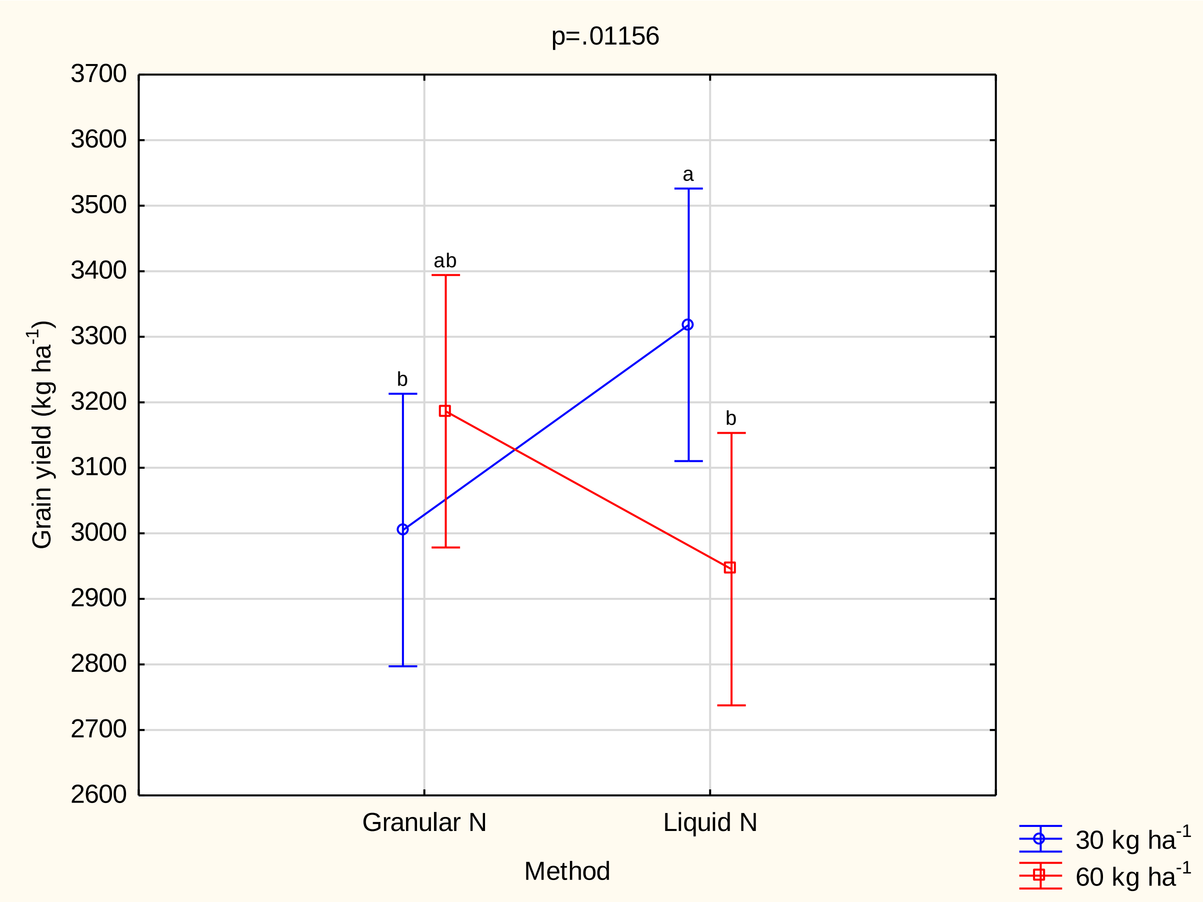

Figure 4.3: Effect of the interaction between the method of N application and N rate of grain yield at Roodebloem in 2014. Different letter above bars indicate significant differences at p = 0.05

At Langgewens, GY was significantly (*p≤0.05*) affected by the N topdressing treatments in 2013. The highest mean GY response (4 239 kg ha^{-1}) was obtained from the application of 30 kg N ha^{-1} LAN+ treatment at Langgewens in 2013 (Table 4.10). This, however, was not significantly different to several other treatments with the exception of 30 kg N ha^{-1} LAN, 30 kg N ha^{-1} granular urea and 60 kg N ha^{-1} LAN.

In 2014, the mean GY ranged between 911 kg ha^{-1} and 1 689 kg ha^{-1} from the control treatment and 30 kg ha^{-1} LAN+ treatments respectively. As shown in Table 4.10, no significant differences were observed between treatments in 2014. The experimental plots were infested by a herbicide resistant ryegrass (*Lolium* spp.) and this probably reduced the effect of the treatment. Agenbag (2012) reported that herbicide resistant ryegrass lowered GY due to suppressed growth and development of wheat plants at Langgewens. In 2015, no significant treatment effect was observed at Langgewens. The lack of GY responses in Langgewens in 2015 could have been due to the lack of sufficient moisture during the critical stages of the crop as this area received very low seasonal rainfall compared to the long-term seasonal rainfall. There was a severe drought in the Swartland sub-region including Langgewens where this study was undertaken. Coetzee (2017) also reported lack of N responses to N fertilisation for canola in 2015 at Langgewens. The author suggested that these conditions prohibited efficiency of N uptake during the critical periods of crop growth.

The evaluation on the effect of N rate at Langgewens showed that there was a significant ($p \leq 0.05$) difference between 30 and 60 kg N ha^{-1} in 2013. As expected, the application of 60 kg N ha^{-1} resulted into a significantly higher mean GY of 3910 kg ha^{-1} compared to application of N topdressings at 30 kg ha^{-1} (3 577 kg ha^{-1}) (Results not shown). Galleto et al. (2017) found that increasing N topdressing rate at tillering increased plant height, TKM, GY but favoured lodging and reduced HLM in Ponta, Paraná, Brazil. No significant N topdressing responses were recorded in 2015. The reason why N rate did not influence GY in 2015 and 2014 is most probably the low rainfall in these two years resulting in the plants not having access to sufficient water to respond to the higher N levels.

Although not significant, the effect of method of N application showed that the application of N topdressings as a liquid offered a slight GY benefit (3 893 kg ha^{-1}) compared to granular applied N topdressings (3 604 kg ha^{-1}) in 2013 and 2014 (1 222 kg ha^{-1} compared to 1162 kg ha^{-1}) (Data not shown). These yield responses from granular and liquid N applications were also observed at Roodebloem. Gooding and Davies (1992) reported that, with leaf damage avoided, foliar urea application increased wheat grain yield and grain protein content when applied before flag leaf emergence. Bhuyan et al. (2012) later reported increases in grain yield of rice with foliar bed applications compared to the conventional application method of nitrogen. When applied early in the season (tillering), it seems that liquid N applications do possess a potential to improve grain yields. However, it remains unclear as to which conditions favour these yield benefits. The challenge is complicated further by the high seasonal variations in climate often found in the Mediterranean areas. The N rate x method of N application interaction was not significant.

4.3.3 Thousand kernel mass (TKM)

At Roodebloem, the highest mean TKM was 35 g from 30 kg N ha^{-1} LAN and UAN treatments followed by 33 g from 60 kg N ha^{-1} LAN in 2013 (Table 4.10). Generally, the 30 kg N ha^{-1} treatments resulted in significantly higher TKM than the 60 kg N ha^{-1} treatments. In 2014, the control treatment resulted into the highest mean TKM (44 g) while the 30 kg N ha^{-1} granular urea and 60 kg N ha^{-1} liquid urea produced the lowest mean TKM (40 g). Hoogmoed et al. (2014), who found that the control treatment had a significantly higher TKM compared to the fertilised plots, reported similar findings. This could be due to the early depletion of moisture reserves resulting from higher vegetative growth and thus increased water stress during grain filling, or the reduction in the storage of water-soluble carbohydrates that serve as the sources of assimilates for translocation during grain fill (Masclaux-Daubresse et al. 2010).

At Langgewens, there were no significant differences between treatments for TKM in 2013 and 2015 (Table 4.10). Statistically significant ($p \leq 0.05$) differences were observed between treatments in 2014. The UAN treatments produced significantly higher TKM values than any of the other treatments. The responses shown by UAN could probably be due to the ability of liquid N to bypass the soil-root system, minimising the risks and losses through leaching (Poulton et al. 1990). Sadaphal and Das (1966) reported that liquid (foliar applied) N applications between flag and ear emergence increased the number of grains and grain weight. Similarly, Veesar et al. (2017) recently found a significant increase in TKM when foliar N was applied at tillering growth stage.

4.3.4 Hectolitre mass (HLM)

In 2013, the HLM values ranged between 73 and 74 kg hl^{-1} as shown in Table 4.10 at Roodebloem. Similarly, in 2014, there was very narrow margin between the treatments in terms of HLM. Table 4.10 shows that the mean highest HLM was 78 kg hl^{-1} while the lowest HLM was 77 kg hl^{-1}. The smaller differences between different N treatments could be an indication that the HLM is affected more by other factors than N fertilisation. Nel et al. (1998) reported that although genetically associated with a cultivar, HLM is largely affected by the growth conditions during the period of grain filling.

At Langgewens, the results of this study showed that HLM was not affected by the N fertiliser topdressing treatment in the 2013 and 2014 growing seasons. The mean HLM values were very similar in the two seasons and ranged between 80 and 82 kg hl^{-1}(Table 4.10). During the 2015 growing season, the HLM was significantly affected by the N treatment and the HLM vakues ranged between 79 and 76 kg hl^{-1} (Table 4.10). To a certain extent, it seems that the lower N rates tended to favour higher HLM at Langgewens in 2015. Varga and Svečnjak (2006) reported that the HLM of different cultivars was improved only at lower N rates compared to higher N rates in studies conducted in Croatia. Maali and Agenbag (2006) mentioned a somewhat similar response, where low N rates tended to result in higher HLM although the differences were not statistically significant.

4.3.5 Plant biomass (PB)

At Roodebloem, the effect of N fertiliser topdressing treatment significantly ($p \leq 0.05$) affected plant biomass in 2014. The highest mean plant biomass was produced from plants that received 60 kg N ha^{-1} granular urea. This was however not significantly different to the mean plant biomass obtained from UAN applied at 30 kg N ha^{-1} (Table 4.10). The responses shown by plants treated with urea could be due to the ability to absorb urea as an intact molecule through the root system by means of specific root transporters (Tan et al. 2000; Kojima et al. 2006). Witte (2010) further mentioned that plants can hydrolyse and utilise urea efficiently due to these urea transporters. On the one hand, the N available from the supply through UAN may have induced higher N uptake and assimilation in these plants, which in turn improved plant biomass. Keys et al. (1978) reported that the amount of ammonium (available in UAN) flux through the photorespiration pathway in the leaves of C_3 plants is five- to ten-times better than that produced from nitrate reduction.

At Langgewens, a significant (*p≤0.05*) fertiliser N topdressing effect was observed at in 2014 growing season. Plants treated with LAN applied at 60 kg N ha^{-1} with an additional spray of Activate N produced the highest plant biomass (Table 4.10). The effect of this treatment was not statistically different to several other treatments although it was statistically different from the control (0 N topdressing), 30 kg N ha^{-1} LAN and 60 kg N ha^{-1} granular urea (Table 4.10). The relatively better responses with LAN 60+ could be attributed to the effect of plant growth -promoting *Rhizobacteria* (PGPRs) on nutrient uptake. Although still a relatively new technology, several authors have reported positive responses of plants inoculated with PGPRs compared to non-inoculated plants (Singh and Kapoor 1999; Egamberdiyeva and Hoflich 2004). These PGPRs promote plant growth through improvement of root development, either directly by affecting the plant metabolism or by promoting other beneficial microorganisms to enhance their actions on the plants (Pérez-Montaño et al. 2014). Rosas et al. (2009) reported increases in grain yields, harvest index and protein content with lower fertilizer doses in plants inoculated with PGPRs compared to non-inoculated plants. Dawwam et al. (2013) also noted significant differences in vegetative growth parameters and photosynthetic pigments coupled with increased N, P, and K concentrations in potatoes.

4.3.6 Harvest index (HI)

A significant (*p≤0.05*) fertiliser N treatment effect on HI was observed in 2014 at Roodebloem (Table 4.10). Plants treated with 30 kg N ha^{-1} with granular urea produced the highest mean HI (0.44) while the lowest mean HI (0.39) resulted from LAN 60+. These responses could be attributed to the efficient assimilation of urea in plant roots, which probably improved NUE.

Gerendás et al. (1998) reported large amounts of urea molecules from the root system compared to the vegetative parts of the plant.

At Langgewens, the results showed that there was no significant effect from the N treatments on HI in both 2014 and 2015 (Table 4.10). In 2014, the application of LAN at 60 kg N ha with Activate N produced the highest HI (0.49), while the highest mean HI (0.37) was obtained from liquid N application at 30 kg N ha^{-1} in 2015.

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Table 4.10: Effect of N fertiliser topdressing on grain yield (GY), thousand kernel mass (TKM), plant biomass (PB) and harvest index (HI) at Roodebloem and Langgewens from 2013 to 2015

		Roodebloem					Langgewens				
Year	Treatment	GY	TKM	HLM	PDM	HI	GY	TKM	HLM	PDM	HI
		kg ha^{-1}	g	kg hl^{-1}	g m^{-2}		kg ha^{-1}	g	kg hl^{-1}	g m^{-2}	
2013	Control	2593	33ab	73b	-	-	3931ab	37	81	-	-
	LAN 30+	2371	32b	74a	-	-	4239a	39	80	-	-
	LAN 60+	2471	32b	74a	-	-	4011ab	39	81	-	-
	Granular LAN 30	2891	35a	74a	-	-	3543b	37	80	-	-
	Granular LAN 60	2455	33ab	74a	-	-	3535b	36	80	-	-
	Granular Urea 30	2706	32b	74a	-	-	3441b	37	81	-	-
	Granular Urea 60	1981	32b	73b	-	-	3897ab	36	81	-	-
	Liquid Urea 30	2448	32b	73b	-	-	3729ab	37	80	-	-
	Liquid Urea 60	3012	32b	74a	-	-	4034ab	36	81	-	-
	Liquid UAN 30	2837	35a	73b	-	-	3596ab	37	80	-	-
	Liquid UAN 60	2746	32b	74a	-	-	4213a	37	80	-	-
	Mean	**2592**	**33**	**74**	**-**	**-**	**3834**	**37**	**81**	**-**	**-**
	Cv (%)	**11.17**	**3.64**	**0.69**	**-**	**-**	**7.33**	**2.82**	**0.65**	**-**	**-**
	P Value	***0.690***	***0.010***	***0.003***	**-**	**-**	***0.027***	***0.610***	***0.249***	**-**	**-**
2014	Control	3318a	44a	78	1064bc	0.41ab	811	39c	81	932b	0.35
	LAN 30+	3132ab	42abc	77	1074bc	0.41ab	1689	39c	81	1104ab	0.45
	LAN 60+	3385a	40c	77	1016bcd	0.39b	1529	40bc	82	1183a	0.49
	Granular LAN 30	3211ab	42abc	78	1042bc	0.42ab	1221	40bc	81	942b	0.35
	Granular LAN 60	3288a	41bc	78	1029bcd	0.40ab	1117	40bc	82	1045ab	0.42
	Granular Urea 30	2889b	40c	78	998bcd	0.44a	1331	39c	82	1072ab	0.44
	Granular Urea 60	3084ab	41bc	77	1170a	0.43a	1081	38c	82	987b	0.38

Values within a column differ significantly at *p = 0.05* if they are followed by different letters, + = Active N followed the LAN at 30 0r 60 kg N ha^{-1}

Table 4.10 Continued from previous page

		Roodebloem					Langgewens				
Year	Treatment	GY	TKM	HLM	PDM	HI	GY	TKM	HLM	PDM	HI
		kg ha^{-1}	g	kg hl^{-1}	g m^{-2}		kg ha^{-1}	g	kg hl^{-1}	g m^{-2}	
2014	Liquid Urea 30	3249a	41bc	78	990cd	0.42ab	1409	38c	81	1004ab	0.39
	Liquid Urea 60	2818b	41bc	78	988cd	0.41ab	1432	38c	81	1109ab	0.45
	Liquid UAN 30	3388a	43ab	78	1092ab	0.43a	1052	42a	81	1010ab	0.4
	Liquid UAN 60	3073ab	41bc	78	942e	0.41ab	996	42a	82	991ab	0.39
	Mean	**3198**	**41**	**78**	**1037**	**0.42**	**1081**	**40**	**81**	**1032**	**0.41**
	Cv (%)	**6.56**	**2.92**	**0.60**	**5.98**	**3.47**	**20.96**	**3.64**	**0.64**	**7.40**	**10.80**
	P Value	***0.011***	***0.001***	***0.100***	***0.050***	***0.029***	***0.610***	***0.010***	***0.100***	***0.042***	***0.088***
2015	Control	3318	35	81	621	0.70	1495	32	79a	575	0.28
	LAN 30+	3317	37	81	649	0.57	1064	33	79a	447	0.31
	LAN 60+	3574	37	80	675	0.47	956	33	78ab	484	0.31
	Granular LAN 30	3464	36	81	593	0.69	1277	32	78ab	581	0.29
	Granular LAN 60	3471	36	80	725	0.47	1309	32	77b	597	0.28
	Granular Urea 30	3569	37	81	700	0.46	1066	32	79a	514	0.29
	Granular Urea 60	3571	37	81	622	0.63	1132	32	78ab	476	0.35
	Liquid Urea 30	3466	37	81	632	0.72	1355	31	76b	560	0.37
	Liquid Urea 60	3569	37	81	700	0.46	1066	32	79a	514	0.29
	Liquid UAN 30	3488	37	81	729	0.58	991	31	78ab	491	0.27
	Liquid UAN 60	3563	37	81	537	0.77	1101	31	76b	528	0.29
	Mean	**3488**	**37**	**81**	**653**	**0.59**	**1164**	**32**	**78**	**524**	**0.30**
	Cv (%)	**2.70**	**2.25**	**0.50**	**10.92**	**18.56**	**15.06**	**2.36**	**1.30**	**10.27**	**11.34**
	P Value	***0.290***	***0.320***	***0.550***	***0.296***	***0.530***	***0.183***	***0.910***	***0.023***	***0.570***	***0.710***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters, + = Active N followed the LAN at 30 0r 60 kg N ha^{-1}

4.3.7 Effect of the interaction between locality and growing season (year)

4.3.7.1 Grain yield (GY)

The effect of interaction between the locality and the season shows that Langgewens produced a significantly higher GY compared to Roodebloem in 2013 (Table 4.11). This could be ascribed to the good seasonal rainfall received at Langgewens in 2013. However, the 2014 and 2015 season showed a significant decrease of 67 and 70% in GY respectively owing to the biological and environmental challenges identified above at Langgewens. Lòpez-Bellido et al. (1996) reported that wheat failed to respond to N when the annual rainfall was low (<450 mm) during the season in studies conducted under Mediterranean conditions in Spain. On the one hand, the higher grain yield at Roodebloem in 2014 and 2015 could probably be a result of better soil carbon and N content recorded at this locality. Agenbag (2012) reported vigorous crop growth, which was probably a result of improved soil organic carbon and N contents. The significantly lower GY responses in 2013 and 2014 could probably be an indication that soil mineral N was lost through leaching and to a lesser extent, denitrification compared to the 2015 season, which resulted into yield penalties.

4.3.7.2 Thousand kernel mass (TKM)

Table 4.11 shows that the interaction between the locality and the season resulted into a significantly higher mean TKM (41 g) at Roodebloem in the 2014 growing season. A significantly lower mean TKM (32 g) was recorded at Langgewens in the 2015 growing season. Lack of sufficient moisture at Langgewens during the September and October months (grain filling stage) probably caused the low TKMs observed at Langgewens in the 2015 growing season. Singh and Malik (1983) confirmed similar findings where the maximum reduction of moisture stress on TKM was recorded when stress levels were induced during flowering to maturity. Jatoi et al. (2011) reported a 21.53% decline in TKM as a result of water stress. The lower TKM responses at Roodebloem in 2013 were probably a result of higher N losses due to leaching and to a certain extent denitrification. The climatic data shows that

August received almost double the amount of rainfall in 2013 compared to 2014. This may have reduced the pre-anthesis assimilates which are later transferred to grain filling (Weng et al. 1982). Iqbal et al. (2012) found that increasing N levels increased TKM in studies conducted in Pakistan, indicating the importance of N for TKM. Chandra et al. (1992) and Maqsood et al. (2002) also reported a linear increase with increasing N levels in TKM, which further confirms that N limitations probably reduced TKM.

4.3.7.3 Hectolitre mass (HLM)

The interaction between locality and season suggests that Langgewens produced significantly higher mean HLM (82 kg hl^{-1}) during the 2014 season (Table 4.11). The lowest mean HLM resulting from this interaction was recorded at Roodebloem in 2013. A study by Nel et al. (1998) on sources of variation on GY and quality of Western and Southern Cape cultivars showed that Moorreesburg produced a larger mean HLM compared to other localities although the mean rainfall in this region was lower during the study period, illustrating inherent ability of this sub-region to produce grain with high HLMs. The lower responses at Roodebloem were probably due to N losses through leaching as was speculated for GY above. It could also be possible that other factors such as temperature and solar radiation probably affected the HLM responses. Hollins et al. (2004) reported that solar radiation and temperature accounted for 62 and 66% year-to-year variance in HLM respectively compared to other climatic factors in rye and wheat studies conducted in Finland. The lower HLM obtained at Langgewens in 2015 could be ascribed to depleted moisture reserves during the critical grain filling stage. Mbave (2013), who reported that water stress imposed on South African wheat cultivars resulted into significantly lower HLM compared to other treatments, confirmed these findings. Lower HLM could also be due to the percentage of small malformed and broken kernels resulting from stress at grain filling (Mirzaei et al. 2011).

4.3.7.4 Plant biomass

Significant locality and season interactions were observed for plant biomass. Langgewens showed significantly lower plant biomass in 2015 (Table 4.11). A shortened season due to early leaf senescence because of drought probably reduced the efficiency of N translocation and remobilization. According to Masclaux-Daubresse et al. (2010) leaf senescence is essential for nitrogen mobilization. The authors argued that delays in leaf senescence prolong photosynthesis that increase grain yield and carbon filling into seeds. The variations in plant biomass responses from this interaction could be a direct combination of soil moisture variations and soil N availability. According to Campbell et al. (1977), N fertility, soil moisture status and other meteorological conditions are the key factors affecting the rate of dry matter accumulation in plants. Ritchie and Johnson (1990) reported that fertiliser N stimulated dry matter accumulation through leaf area increase and improved plant transpiration. It could also be speculated that relatively lower soil N in 2015 (Table 4.4) compared to that of the 2014 season probably contributed to poor dry matter accumulation at Langgewens in 2015. Ismail et al. (1999) found that water stress reduced the yield components of wheat irrespective of the growth stage exposed to water stress. Gabaret et al. (1998) reported that the amount of precipitation and its distribution play a major role on the effect of N fertilisation on dry matter production and the effect varied between years. On the other hand, McDonald (1992) found that addition of N fertiliser up to 100-150 kg ha^{-1} increased dry matter production in South Australia.

4.3.7.5 Harvest index (HI)

Harvest index is the ratio of grain yield to the plant biomass (Donald and Hamblin 1976). Factors that affect N uptake, assimilation, and translocation would probably affect the HI. The interaction between season and locality showed that no significant difference between Langgewens and Roodebloem occurred in 2014 but in 2015 Roodebloem showed a significantly better HI. The lack of significantly different responses from this interaction in 2014 could be due to similar responses observed for plant dry matter in the same year, which may confirm the relationship between dry matter partitioning and harvest index. Nanja Reddy et al. (2003) reported a positive relationship between plant dry matter and harvest index in sunflower (*Helianthus annuus* L.) genotypes. The authors proposed that increasing biomass production at post-anthesis combined with increased biomass partitioning to sink through sink properties could be one approach used to improve grain yields. The higher and lower responses shown in Table 4.11 for Roodebloem and Langgewens (respectively) in 2015 could be ascribed to the contrasting seasonal rainfall received by these two localities in 2015. Chakwizira et al. (2016) reported increases in both harvest index and nitrogen harvest index with increases in moisture supply both at dryland and irrigation conditions in maize, explaining the higher harvest index at Roodebloem compared to Langgewens.

4.3.7.6 Water use efficiency (WUE)

Table 4.11 shows that the interaction between season and locality resulted in significantly ($p \leq 0.05$) higher WUE at Roodebloem in 2014 although this was not statistically different to 2015 at the same locality (Table 4.11). The crop responses to this interaction could be ascribed to both soil moisture status and soil N mineralization. Quemada (2004) reported that soil N supply through soil organic matter mineralization was governed by soil moisture status. In a more recent study, Quemada and Gabriel (2016) reported that crop water shortages induce yield and biomass reduction including poor N uptake, which could explain the poor WUE responses at Langgewens in 2015. Ngezimana (2012), who found that Roodebloem produced significantly better WUE compared to other localities including Langgewens reported similar responses. The author associated these variations with rainfall distribution and intensity.

4.3.8 Grain protein content (GPC) and falling number (FN)

The study revealed that there was no significant effect of N fertiliser topdressing treatments on GPC and FN at both localities. The trends however, suggest that GPC increased with increasing N rate for both localities as shown in Table 4.12. Maali and Agenbag (2006) reported a linear increase in GPC with increasing N rates at Langgewens. The GPC values varied between 11 and 12% at Roodebloem. At Langgewens, the GPC ranged between 13 and 15%. The analysis of GPC at Langgewens in 2015 showed relatively higher GPC compared to the other years, which probably resulted into the higher means shown above. This could probably be a result of low grain yield under soil moisture stress in 2015. Grain protein content is known to increase under low yield conditions, while the opposite happens when grain yield is high. Fowler (2003) and Casagrande et al. (2009) reported negative correlation between grain yield and GPC. At the same time, Ransom et al. (2016) who reported low grain yield and elevated levels of grain protein (>160 g kg^{-1}) in locations that experienced lower than average rainfall in USA confirmed these findings.

Table 4.11: Effect of interaction between locality and season on grain yield (GY), thousand kernel mass (TKM), hectolitre mass (HLM), plant biomass (PDM), harvest index (HI) and water use efficiency (WUE) for Roodebloem and Langgewens

Locality	**Year**	**GY (kg ha^{-1})**	**TKM (g)**	**HLM (kg hl^{-1})**	**PDM (g m^{-2})**	**HI**	**WUE (kg grain mm water^{-1})**
Roodebloem	2013	2624d	33d	74d	-	-	8.69c
Langgewens	2013	3835a	37c	81b	-	-	10.95b
Roodebloem	2014	3141c	41a	78c	1038a	0.41b	13.63a
Langgewens	2014	1266e	40b	82a	1033a	0.41b	4.87e
Roodebloem	2015	3506b	37c	81b	652b	0.64a	13.52a
Langgewens	2015	1151e	32e	78c	525c	0.31c	6.56d
Mean		**2587**	**36**	**79**	**812**	**0.44**	**9.71**
Cv (%)		**44.13**	**9.86**	**3.76**	**32.42**	**31.61**	**37.39**
P Value		***0.000***	***0.000***	***0.000***	***0.015***	***0.000***	***0.000***

Values within a column differ significantly at *p = 0.05* if they are followed by different letters

The responses of FN did not show any clear trends at Roodebloem, however, to a lesser extent, there is a gradual increase of FN with increases of N at Langgewens (Table 4.12). Gooding et al. (1986) reported increased FN due to N fertilisation and the authors concluded that this was probably due to delaying crop grain maturity. Nitrogen fertilizer can increase or decrease FN (Stewart and Dyke 1993), however, its effect is lower than the effects of cultivar and climatic conditions (Smith and Gooding 1999).

Table 4.12: Effect of N fertiliser topdressing treatments on mean grain protein content (GPC) and falling number (FN) at Roodebloem and Langgewens (2013-2015)

	Roodebloem		**Langgewens**	
Treatment	**GPC (%)**	**FN (s)**	**GPC (%)**	**FN (s)**
Control	11	356	13	382
LAN 30+	11	322	13	396
LAN 60+	12	352	14	403
Granular LAN 30	11	392	14	390
Granular LAN 60	12	343	15	367
Granular Urea 30	11	360	13	393
Granular Urea 60	12	379	13	385
Liquid Urea 30	11	333	14	382
Liquid Urea 60	11	354	14	400
Liquid UAN 30	11	369	13	387
Liquid UAN 60	12	356	14	410
Mean	**11**	**356**	**14**	**390**
Cv (%)	**4.44**	**5.54**	**4.94**	**3.03**
P value	***0.990***	***0.950***	***0.970***	***0.980***

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate

+ = LAN followed by foliar application of Activate N

4.3.9 Multiple regression analysis for Roodebloem and Langgewens

Table 4.13 shows the multiple regression analysis of different variables evaluated in this study. From the table, it is shown that TKM, PDM and HI were excluded from the analysis due to severe multi-collinearity effect. The model shows that grain yield was significantly affected by HLM and WUE and the two variables accounted for 98% of the variation (Adjusted R^2 = 0.98). According to this model, a one unit increase in HLM will result in 16% (b* = 0.16) increase in grain yield if WUE is held constant. Similarly, if WUE increases by one unit, that will cause an increase of more than 100% (b* = 1.01) if the HLM is controlled. This generally explains the significant role that seasonal rainfall probably played in variations observed within the locality and between the two localities.

Table 4.13: Multiple regression analysis of hectolitre mass (HLM), water use efficiency (WUE), thousand kernel weight (TKM), plant biomass (PB) and harvest index (HI) on grain yield at Roodebloem and Langgewens combined

	Regression Summary for Dependent Variable: Grain yield R= .99194213 R^2= .98394920 Adjusted R^2= .98370035						
N=132	b*	Std.Err.	b	Std.Err.	t(129)	p-value	# times in best 20 models
Intercept			-7421.08	506.8212	-14.6424	0.000000	
HLM	0.162377	0.011379	89.84	6.2958	14.2699	0.000000	5
WUE	1.011174	0.011379	264.33	2.9746	88.8635	0.000000	5
TKM	Excluded						5
PB	Excluded						5
HI	Excluded						5

4.4 Conclusions

The study showed that the effect of N topdressing treatment was not consistent across the studied parameters over the three-year period at both localities. Although not significant, the application of liquid N topdressing (spray) performed better than granular applied N topdressing in terms of GY in two (2013 and 2014) of the three study years for both localities. Grain yield increased with increasing N level at Langgewens in the 2013 growing season. The N rate x method of application interaction indicated that there was a significant GY increase when N was applied as a liquid spray at 30 kg ha^{-1} compared to either granular N applied at 30 kg N ha^{-1} or liquid N applied at 60 kg N ha^{-1} . The effect of locality showed that Roodebloem produced significantly better GY than Langgewens. This was probably caused by resistant ryegrass that competed with wheat at Langgewens in 2014 and a severe drought in 2015. There was no clear trend observed for TKM, HLM, PDM and HI due to the effect of N fertiliser treatment for both localities. TKM and HLM varied largely due to season and locality. Seasonal rainfall in both localities was a major contributing source of variation for TKM and HLM both within the locality and between the two localities. The N topdressing treatment did not significantly influence GPC and FN, although trends tended to favour higher N rate for GPC. The multiple linear regression analysis showed that WUE and to a lesser extent, HLM significantly influenced grain for the two localities.

4.5 References

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Chapter 5

Effect of granular and liquid applied nitrogen on yields and yield parameters of spring wheat (*Triticum aestivum* L.) under controlled glasshouse conditions

Abstract

A glasshouse pot experiment was conducted to study the effect of soil and foliar application of nitrogen (N) on yield and yield parameters of spring wheat (*Triticum aestivum* L.) during three growing seasons (2013, 2014 and 2016). The randomized complete block design experiment with 10 treatments was replicated six times. The treatments were LAN (28%) and urea (46%) applied as granular N topdressing and UAN (32%) and liquid urea (46%) solution applied as liquid sprays. The treatments were applied at N rates equivalent to 30 and 60 kg N ha^{-1} at mid-tillering following an application of LAN at 30 kg N ha^{-1} at sowing. The control treatment received 0 kg N ha^{-1}. To analyse the effect of method of application (granular and liquid) and N rate (30 and 60 kg N ha^{-1}), data was extracted from the selected treatments and analysed as a 2x2 factorial design. Nitrogen treatments significantly influenced plant biomass-, number of ears-, mass of ears- and grain yield pot^{-1} in all seasons. Harvest index was significantly affected by the N treatment in 2014 and 2016. The responses showed that 60 kg N ha^{-1} promoted positive and significantly ($p \leq 0.05$) higher yields and yield parameters compared to 30 kg N ha^{-1} and that liquid applied N was superior compared to granular N throughout the study. The multiple linear regression analysis showed that plant biomass and harvest index significantly affected grain yield. The N fertiliser treatment however, did not have any significant effect on grain protein content and falling number.

Keywords: Granular applied nitrogen, limestone ammonium nitrate (LAN), liquid applied nitrogen, UAN solution, urea solution

5.1 Introduction

Liquid application of nitrogen (N) is becoming a common practice world-wide due to benefits associated with the practice. There are however, mixed results on liquid (foliar) nutrient applications, with some of the results showing positive effects, others negative and sometimes no responses depending on crop species and nutrients applied (Fageria et al. 2009) . Gooding and Davies (1992) reported increased grain yield after foliar N applications and the effect was more profound when N was applied before flag leaf emergence or when N availability was limiting. When compared to direct N applications to the soil, losses of N through denitrification and leaching were reduced with application of urea solution (Balasubramanian et al. 2004).

In wheat, a foliar N application resulted in higher grain protein concentration than granular soil applied fertiliser at late growth stages (7 to 10 weeks after seeding) (Alkier et al. 1972; Strong 1982). Work by Bly and Woodard (2003) showed that grain protein content and yield were inversely related ($R^2 = 0.57$) in plots without foliar N, and 9 out 12 sites had significant grain protein concentration responses to foliar N application. The authors reported that postpollination foliar N increased protein concentration 70% of the time when yield goal was exceeded compared with only 23% when it was not. Findings by Varga and Svečnjak (2006) indicated that there is a potential positive benefit in use of late urea spraying for improved grain yields in winter wheat if previous N applications were suboptimal for maximum yield potential.

From the above, it is clear that there are benefits associated with applying N fertiliser to the leaves to supplement earlier soil applied N. Several authors have reported that the integrated use of granular (soil applied) N at sowing and liquid sprays during the season is an effective and economical approach (Khaskhely 1991; Abdi et al. 2002) to improve yields and yield components (Grewel and Mittal 1982; Shah and Saeed 1990; Emam and Borjian 2000). In South Africa, generally, very few studies have been conducted on the integration of liquid N spray application as means to improve either plant growth, grain yield or grain quality parameters. The objective of this study was to evaluate the effect of applying N fertiliser topdressing using granular N fertiliser sources applied to the soil versus applying liquid N sprays on grain yields, grain quality and yield parameters of wheat (*Triticum aestivum* L.) under controlled glasshouse conditions.

5.2 Materials and method

An experiment was conducted under glasshouse conditions for three years (2013, 2014 and 2016) at Welgevallen Experimental Farm, Department of Agronomy, University of Stellenbosch, Stellenbosch, South Africa (33°56'33"S, 18°51'56"E, 136 m.a.s.l.). The experiment was designed as a randomized complete block design with 10 treatments replicated six times. Wheat cultivar SST 027 was planted in pots filled with field soil as a growing medium. The area of the pots was 0.03 m^2. About 0.32 g pot^{-1} of Limestone Ammonium Nitrate (LAN 28% N) was applied in all the pots at sowing with the exception of the control (0 N). The allocation of treatments is shown in Table 5.1. The chemical properties of the soil used as a growing medium is shown in Table 5.2. After sowing, the growing medium was kept moist using tap water. After germination, pots were irrigated manually to field capacity with a nitrogen free balanced standard nutrient solution (Table 5.3) every three to four days depending on the moisture status of the soil. To determine the field capacity, a measuring cylinder filled with water was used to apply water in the first pot and the water was allowed to move freely and the action was stopped as soon as the water emerged at the bottom of the pot. The measured amount was then used as a benchmark for field capacity and was applied in all other pots. The glasshouse was set at a 10/25°C night/day temperature throughout the study period.

Two weeks after emergence, plants were thinned from five plants to three plants pot^{-1}. To evaluate the effect of N topdressing, the treatments were applied once at tillering (Zadoks GS

21) (Zadoks et al. 1974). For foliar urea ammonium nitrate (UAN) (32%) treatment, the solution was prepared by mixing 88.75 and 177.5 ml of UAN solution with 500 ml of water. To prepare the liquid urea solution, 81.5 and 163 g of urea granules were dissolved in 500 ml of water. The liquid N spray treatments were applied during the morning hours of the day using a pot-spraying apparatus at a pressure of 2 kPa and with a delivery rate of 400 litres of water ha^{-1} obtained from the Department of Agronomy, University of Stellenbosch. These treatments were applied in the form of overhead sprays on top of the plant leaf surface although a certain amount of the liquid dropped on top of the soil surface; hence the use of the term liquid N topdressing is more prefered than foliar application. Since the crop was still at the tillering growth stage (no full canopy), a certain amount of the liquid N fertilizer applied was taken up by the leaves while some was taken up by the roots from the soil. The aim of the study was to evaluate the effect of applying N as granules broadcast on the soil surface versus application of N as liquid sprays. The N solutions were prepared to apply N amounts equivalent to those applied through the granular fertilisers. The granular urea treatments were applied by spreading urea granules on the soil surface of the pot using 0.19 and 0.38 g of urea per pot. Limestone ammonium nitrate was applied using 0.32 and 0.64 g pot^{-1}. The application rates were selected to give N levels approximately equal to 30 and 60 kg N ha^{-1} on an area basis. In one treatment, LAN application at 30 N ha^{-1} equivalent was followed by spraying plants with Activate N (see Chapter 4) and this treatment is denoted as LAN 30+ in text. The order of activities conducted in the experiment is shown in Table 5.4. The planting dates in the glasshouse depended largely on the availability of working space. For this reason, the experiment could not be repeated at similar times of the year.

At physiological maturity, above ground plant material was cut off at stem base and samples of each treatment were collected, dried until constant weight (60°C for 48 hours), weighed and threshed. Plant biomass pot^{-1} (PBPP), number of ears pot^{-1} (NEPP), mass of ears pot^{-1}(MEPP), grain yields pot^{-1} (GYPP) were measured and the harvest index (HI) was calculated by dividing the total biomass (above ground) by the total grain yield. Grain protein content was determined at the Welgevallen Experimental Farm at the University of Stellenbosch using the Near-Infrared Reflectance method for protein in wheat flour AACC Method 39-11 (American Association of Cereal Chemists 2000a). The falling numbers were determined following the standard procedure according to the American Association of Cereal Chemists (2000b) at the Welgevallen Experimental Farm.

Table 5.1: Nitrogen topressing treatments in the glasshouse experiment 1 in 2013, 2014 and 2016

Treatment	Method	Sowing (LAN) ($g\ pot^{-1}$)	Tillering
Control	0	0	0
LAN 30+*	0	0.32	0.32 $g\ pot^{-1}$
LAN 30	Granular	0.32	0.32 $g\ pot^{-1}$
LAN 60	Granular	0.32	0.64 $g\ pot^{-1}$
Urea 30	Granular	0.32	0.19 $g\ pot^{-1}$
Urea 60	Granular	0.32	0.39 $g\ pot^{-1}$
Urea 30	Liquid	0.32	81.5 g 500 ml^{-1}
Urea 60	Liquid	0.32	163 g 500 ml^{-1}
UAN 30	Liquid	0.32	88.75 ml 500 ml-1
UAN 60	Liquid	0.32	177 ml 500 ml^{-1}

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate, *LAN 30+ was substituted with UAN 90 in 2016

Analysis of variance (ANOVA) was performed using the general linear model (GLM) Procedure of Statistica 13.2 to test for differences between treatments for all parameters (StatSoft Inc. Tulsa, OK, USA). Means were separated using the Fisher's protected least significant difference (LSD) test at $p \leq 0.05$. In cases where residuals were not normally distributed, the Kruskal-Wallis test was used as a non-parametric test to confirm the results of the ANOVA. In cases where Levene's test for homogeneity of variances indicated heterogeneous variances, the LSD test was replaced with the Games-Howell multiple comparison procedure. The effect of the method of N application (granular or liquid) and N rate was analysed by extracting data from treatments denoted as granular and liquid (Table

3.8). This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rate levels; 30 and 60 kg N ha^{-1}) factorial.

The variance estimation, precision and comparison (VEPAC) package of Statistica was used for statistical analyses. To analyse the grain protein content, samples per replicate were too small to meet the minimum requirements (in grams) for the analysis of protein content and therefore, samples from the six replicates per treatment were combined to represent the treatment and the grain samples from 2013, 2014 and 2016 were used as replicates. Due to severe fungal disease infestation on young wheat plants in 2015, the 2015 experiment was abandoned, which explains the missing year.

Multiple linear regression analysis was performed for all the tested variables and grain yield was the dependant variable to assess which variable/s contributed significantly to the grain yield and to further evaluate the relationship between the different variables. Where there was severe multi-collinearity among the input variables, the best four input variables were selected using the best subsets procedure and variables were then reduced to the best three or two input variables.

Table 5.2: Soil characteristics of the Welgevallen soils used in the study for 2013, 2014 and 2016

	pH	Resist.	H^+	P (Bray II)	K	Na	K	Ca	Mg	Cu	Zn	Mn	B	C	Total N	S
Year	**KCl**	**Ohm**	**cmol kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**		**cmol(+) kg^{-1}**				**mg kg^{-1}**			**(%)**		**mg kg^{-1}**
2013	4.8	1420	0.72	89	112	0.06	0.22	2.3	0.31	3.8	3.8	112	0.2	0.9	0.06	11.2
2014	4.7	1120	0.86	126	91	0.05	0.23	1.89	0.33	2.2	3.4	41.5	0.1	0.87	0.06	13.31
2016	4.9	1280	0.68	90	111	0.04	0.25	3.08	0.51	1.9	3.58	50.37	0.24	0.8	0.05	7.8

Table 5.3: The chemical composition of the nutrient solution applied on wheat plants in the glasshouse in 2013, 2014 and 2016

pH (KCl)	EC	Na	K	Ca	Mg	Fe	Cl	CO	HCO	SO	B	Mn	Cu	Zn	P	NH-N	NO-N
	mS.m	mg.l															
6.1	147.3	14.7	280.5	160	52.2	1.46	25.6	0	57	693	0.52	0.16	0.11	0.18	82.6	0.47	6.13

Table 5.4: The dates of different activities in the glasshouse from 2013, 2014 and 2016

Sowing	N Treatment	Harvesting
09 Aug. 2013	16 Sep. 2013	02 Feb. 2014
24 Aug. 2014	30 Sep. 2014	12 Feb. 2015
25 Apr. 2016	20 May 2016	27 Sep. 2016

5.3 Results and discussion

According to the analysis of variance (ANOVA), N fertilizer topdressing treatment significantly (*$p \leq 0.05$*) affected plant biomass pot^{-1} (PBPP), number of ears pot^{-1} (NEPP), mass of ears pot^{-1} (MEPP) and grain yield pot^{-1} (GYPP) in all the study years with the exception of the harvest in 2013 (Table 5.5).

The studied parameters responded significantly to N rate except for HI in 2013, 2014 and 2016, and plant biomass and number of ears in 2014. (Table 5.6). The method of application significantly affected plant biomass, number of ears, mass of ears and grain yield in 2013, while the number of ears and grain yield were the only two parameters that were significantly affected by the method of application in 2014. In 2016, the effect of method of application almost showed a negligible effect and the harvest index was the only tested parameter that responded significantly to the effect. The interaction between N rate and method generally showed no significant responses except for grain yield and harvest index that were significantly affected in 2016.

After using the three years of the study data as replications, the analysis of variance showed that grain protein content (GPC) and the falling number (N) were not significantly affected by the N treatment (Anova not shown).

Table 5.5: Summary of the Anova on the effect of N treatment on plant biomass pot^{-1} (PBPP), number of ears pot^{-1} (NEPP), mass of ears pot^{-1} (MEPP), grain yield pot^{-1} (GYPP) and the harvest index (HI) in 2013, 2014 and 2016 growing seasons

	Source		
	N treatment		
Variable	**2013**	**2014**	**2016**
Plant biomass	*	*	*
No. of ears	*	*	*
Mass of ears	*	*	*
Grain yield	*	*	*
Harvest index	ns	*	*

*Significant at 0.05 probability level

Table 5.6: Summary of the Anova on the effect of N rate (R), method of application (M) and method x N rate interaction on plant biomass pot^{-1} (PBPP), number of ears pot^{-1} (NEPP), mass of ears pot^{-1} (MEPP), grain yield pot^{-1} (GYPP) and the harvest index (HI) in 2013, 2014 and 2016 growing seasons

		Source		
Year	**Variable**	**R**	**M**	**M x R**
2013	Plant biomass	*	*	ns
	No. of ears	*	*	ns
	Mass of ears	*	*	ns
	Grain yield	*	*	ns
	Harvest index	ns	ns	ns
2014	Plant biomass	*	ns	ns
	No. of ears	*	ns	ns
	Mass of ears	*	*	ns
	Grain yield	*	*	ns
	Harvest index	ns	ns	ns
2016	Plant biomass	ns	ns	ns
	No. of ears	ns	ns	ns
	Mass of ears	*	ns	ns
	Grain yield	*	ns	*
	Harvest index	ns	*	*

*Significant at 0.05 probability level
ns – not significant at 0.05 probability level

5.3.1 Plant biomass pot^{-1} (PBPP)

The results showed that wheat plants sprayed with UAN and urea solutions at 60 kg N ha^{-1} significantly (*p≤0.05*) improved plant biomass compared to other treatments in 2013. The lowest mean PBPP was obtained in plants that did not receive any N fertiliser (Control) (Table 5.7). Although LAN 60 produced higher mean PBPP in 2014, this was not significantly different to several other treatments particularly the liquid applications. The results show that there was a tendency of plants treated with liquid urea to show poor plant biomass compared to other treatments in 2016. This could be ascribed to the accumulation of toxic urea amounts in the leaves, which probably altered the physiological processes responsible for plant dry matter accumulation. Krogmeier et al. (1989) reported that after foliar feeding with urea, there was a noticeable increase in amount of toxic urea in the leaves of soybean.

Although the objective of the study was not to compare years (season), the responses of plants between the different years showed that there was a better response in 2014 compared to other years and this did not apply only to PBPP but to other studied parameters. This could be ascribed to higher soil P content (126 mg kg^{-1} in 2014) versus 112 mg kg^{-1} (2013) and 90 mg kg^{-1} (2016) which induced higher plant biomass and other studied parameters in 2014 compared to the two other years. Harfe (2017) reported that the application of N and P fertilizers significantly ($p < 0.01$) increased plant height, shoot dry weight at physiological maturity, and tiller number per plant in studies conducted in Ethiopia. The author found that grain and straw N and P contents and their uptakes were strongly and positively correlated with applied N and P respectively. Phosphorus is second only to nitrogen in importance as an essential crop nutrient and it is critical for plant growth, especially in the early jointing stage and for enhancing grain yield and yield components (Römer and Schilling 1986).

The effect of N rate on plant biomass is shown in Table 5.8. Plant responses to N rate showed that 60 kg N ha^{-1} produced significantly ($p \leq 0.05$) higher mean plant biomass compared to N applications at 30 kg ha^{-1} in 2013 and 2014. This could be due to the increase in photosynthetic rate and higher leaf area, which was promoted by sufficient N reserves early in the season. Morgan (1988) reported that application of N in the early growing stages of the crop enhances dry matter accumulation through tiller number and larger photosynthetic surface area. Kumar and Sharma (1999) and Gholami et al. (2011) also reported that dry matter production increased significantly in wheat when the level of N applied was increased, which confirmed these findings.

Extracting data sets to evaluate the effect of the method of N application showed that, applying N as a liquid spray produced significantly ($p \leq 0.05$) higher PBPP in 2013. Liquid N applications showed a 20% advantage over the conventional methods of applying N in 2013 (Table 5.9). Although the differences were not statistically significant in 2014, the results show that liquid N applications resulted in relatively better dry mass accumulation compared to granular N applications. This could be due to large fractions of leaf N in the photosynthetic enzymes, which resulted in increased leaf photosynthetic rates (Millard and Catt 1988), and eventually high dry mass accumulation. Kumari (2011) reported increases in plant biomass from 2.96 g under low N to 4.62 g under high N supply during the vegetative phase and the author concluded that the increased PDM was due to greater allocation of dry matter to shoots. In contrast, conditions of low N availability probably decreased leaf size due to reduced cell number causing asymmetrical cell division, stopping of cell division, reducing cell flux and small mature cell size that subsequently lead to reduced cell elongation (McAdam et al. 1989; Fricke et al. 1997; Jovanovic et al. 2004). Maitlo et al. (2006) reported significant increases in plant dry weight with 75 kg N ha^{-1} soil application at sowing and 2.5% foliar application of urea solution at early heading in studies conducted in Pakistan.

5.3.2 Number of ears per plant (NEPP)

The results of this study showed that topdressing plants with LAN, UAN, Urea liquid and urea solid, all applied at 60 kg N ha^{-1} produced plants with significantly higher NEPP compared to other treatments (Table 5.7) in 2013. The control treatment responded poorly in terms of NEPP in 2013. The responses suggests that, the N effect was largely influenced by application rate at tillering as all the plants that received 60 kg N ha^{-1} responded better compared to plants that were top-dressed with 30 kg N ha^{-1}. In 2014, the trend was somewhat similar to that observed in 2013. This could be ascribed to the availability of assimilates from the flag leaf under high N supply during the period just before flowering to late reproductive stage (Fischer 1985). The availability of N assimilates probably influenced the number of fertile florets during the anthesis phase (Kumari 2011).

It could also be argued that these treatments were instrumental during the leaf primordia phase, the spikelet primordia phase and the floret primordia phase (González et al. 2011). Lack of sufficient assimilates during these phases could lead to floret primordia that fails to develop fully as a result of floret death when spike growth is at maximum rate (Kirby 1988, Siddique et al. 1989).

The effect of N application rate on NEPP indicates that topdressing spring wheat with 60 kg N ha^{-1} was superior to topdressing at N rates of 30 kg ha^{-1}. Table 5.8 shows that 60 kg N ha^{-1} (8 ears pot^{-1}) resulted in the production of two extra ears pot^{-1} compared to 30 kg N ha^{-1} (6 ears pot^{-1}) in 2013. In 2014, 60 kg N ha^{-1} produced significantly higher ($p \leq 0.05$) NEPP (9 ears pot^{-1}) compared to 30 kg N ha^{-1} (8 ears pot^{-1}). No significant N rate effect was found in this study in 2016. Alazmani (2014) reported that increasing N rate from 0 – 225 kg ha^{-1} significantly increased the number of spikes and other parameters in barley cultivars. The plant responses could also be associated with tiller formation and survival that was influenced by the availability of N early in the season (Langer 1966). Bulman and Hunt (1988) reported that high number of ears produced through increasing or timely application of N to promote tillering and tiller survival could increase or reduce yield depending on plant response.

Table 5.7: Effect of N fertiliser topdressing treatments on plant biomass per pot (PBPP), number of ears per pot (NEPP), mass of ears per pot (MEPP), grain yield (GYPP) and harvest index (HI) under glasshouse conditions in 2013, 2014 and 2016

Year	N treatment	PBPP	NEPP	MEPP	GYPP	HI
		g		g		
2013	Control	7.33e	4d	6.55f	4.67f	0.63
	LAN 30+	16.67d	5c	11.92e	8.72e	0.47
	Granular LAN 30	17.33d	6bc	12.15e	8.33e	0.49
	Granular LAN 60	24.50bc	8a	15.52d	11.73bcd	0.48
	Granular Urea 30	17.33d	6bc	11.97e	8.98c	0.52
	Granular Urea 60	26.00b	8a	17.68c	12.67abc	0.49
	Liquid Urea 30	22.00c	6b	16.20d	10.92cd	0.50
	Liquid Urea 60	31.67a	9a	21.12a	14.25ab	0.46
	Liquid UAN 30	18.00d	6bc	13.23e	9.27de	0.52
	Liquid UAN 60	30.00a	9a	19.28b	14.95a	0.50
	Mean	**21.08**	**7**	**14.56**	**10.45**	**0.51**
	Cv (%)	**34.44**	**25.42**	**29.32**	**29.57**	**10.53**
	P Value	***0.000***	***0.031***	***0.000***	***0.006***	***0.233***
2014	Control	22.55d	4c	8.31d	6.08f	0.28d
	LAN 30+	41.73bc	8b	22.00b	14.92d	0.37bc
	Granular LAN 30	44.08abc	8b	21.73b	15.42cd	0.35bcd
	Granular LAN 60	50.27a	9ab	24.08ab	17.67ab	0.35bcd
	Granular Urea 30	38.50c	7c	17.08c	12.58e	0.33cd
	Granular Urea 60	39.58c	9ab	23.53ab	17.42abc	0.46a
	Liquid Urea 30	44.15abc	8b	25.40a	16.92bcd	0.39abc
	Liquid Urea 60	48.72a	10a	25.42a	16.67bcd	0.34bcd
	Liquid UAN 30	43.53abc	8b	21.20b	15.58cd	0.36bc
	Liquid UAN 60	48.40ab	10a	26.30a	19.33a	0.42ab
	Mean	**42.15**	**8**	**21.51**	**15.26**	**0.37**
	Cv (%)	**18.75**	**21.34**	**24.91**	**24.26**	**13.62**
	P Value	***0.010***	***0.010***	***0.010***	***0.018***	***0.012***
2016	Control	22.67bc	4bc	11.49bc	9.18cde	0.41c
	Granular LAN 30	21.92bc	4bc	12.69bc	9.68cd	0.44bc
	Granular LAN 60	25.59ab	5ab	14.83ab	11.68bc	0.46bc
	Granular Urea 30	21.47bc	5ab	13.71ab	10.90bcd	0.51b
	Granular Urea 60	21.14bc	4bc	12.40bc	8.87de	0.42c
	Liquid Urea 30	14.32d	3c	8.87c	6.60e	0.46bc
	Liquid Urea 60	18.80cd	4bc	11.66bc	8.82de	0.47bc
	Liquid UAN 30	24.15b	5ab	13.55ab	11.37bcd	0.47bc
	Liquid UAN 60	24.79b	5ab	19.51a	17.62a	0.74a
	UAN 90	29.91a	6a	18.25a	13.15b	0.44bc
	Mean	**22.48**	**5**	**13.70**	**10.79**	**0.48**
	Cv (%)	**18.54**	**18.89**	**23.2**	**28.04**	**19.7**
	P Value	***0.031***	***0.010***	***0.020***	***0.010***	***0.010***

Means within a column in each year differ significantly at p = 0.05 if they are followed by different letters

The effect of the method of application showed that liquid N application was significantly (*p≤0.05*) better than granular N applications in the production of ears in 2013. Table 5.9 shows that the application of N as a liquid spray produced on average eight ears pot^{-1} compared to seven ears pot^{-1} produced by plants top-dressed with granular N. Sarandón and Gianibelli (1990) reported increased number of ears per square meter due to foliar urea applications at tillering and the authors argued that N supply at this stage favoured the survival of tillers hence increased number of ears, which was also confirmed by Scott et al. (1977) and Power and Alessi (1978). In 2014 and 2016 there were no significant differences between granular and liquid applied N topdressings.

Table 5.8: Effect of N rate on plant biomass per pot (PBPP), number of ears per pot (NEPP), mass of ears per pot (MEPP), grain yield per pot (GYPP) and harvest index (HI)

Year	N rate (kg ha^{-1})	PBPP (g)	NEPP	MEPP (g)	GYPP (g)	HI
2013	30	18.67a	6a	13.39a	9.37a	0.51
	60	28.04b	8b	18.40b	13.40b	0.48
	Mean	**23.35**	**7**	**15.89**	**11.39**	**0.50**
	Cv (%)	**28.37**	**20.20**	**22.29**	**25.03**	**4.28**
	P value	***0.000***	***0.000***	***0.000***	***0.000***	***0.213***
2014	30	42.57a	8a	21.35a	15.13a	0.36
	60	46.74b	9b	24.83b	17.77b	0.39
	Mean	**44.65**	**8**	**23.09**	**16.45**	**0.37**
	Cv (%)	**6.60**	**8.32**	**10.66**	**11.35**	**5.66**
	P value	***0.033***	***0.009***	***0.001***	***0.000***	***0.085***
2016	30	20.46	4	12.21a	9.64a	0.47
	60	22.58	4	14.60b	11.75b	0.52
	Mean	**21.52**	**4**	**13.40**	**10.69**	**0.50**
	Cv (%)	**6.97**	**0.00**	**12.61**	**13.95**	**7.14**
	P value	***0.155***	***0.811***	***0.024***	***0.041***	***0.661***

Means within a column in each year differ significantly at *p = 0.05* if they are followed by different letters

5.3.3 Mass of ears per plant (MEPP)

The mass of ears was significantly (*p≤0.05*) affected by the N fertiliser treatments in all the years of the study (Table 5.7). It is clear from the results that liquid applications of N significantly (*$p \leq 0.05$*) improved mass of ears in this study in 2013 but to a lesser extent in 2014 and 2016. In 2016, the liquid urea application showed inexplicable low values. Studies by Gooding and Davies (1992) showed that about 70% of the applied foliar N was recovered in foliar tissues. This indicates higher N uptake of liquid applied N fertilisers compared to granular applied N. Liquid (foliar) fertilisation assist in direct uptake of nutrients and requires low consumption of energy and the process is less dependent on environmental factors (Roy et al. 2013).

The effect of N rate showed that applications of 60 kg N ha^{-1} significantly improved the MEPP in all three years of this study (Table 5.8). A superior mean mass of 18.4 g pot^{-1} was produced following the application of 60 kg N ha^{-1} at tillering compared to 13.39 g pot^{-1} found in plants top-dressed with N at a rate of 30 kg ha^{-1}. Hussain et al. (2006) reported that increasing the N level from 50 to 200 kg ha^{-1} significantly increased ear mass compared to 0 kg N ha^{-1}. Similarly, Ibramim et al. (2014) found that increasing N rate significantly increased mass of ears in studies conducted on wheat in Egypt.

Applying N as either granular or liquid at tillering triggered significant (*$p \leq 0.05$*) responses in spring wheat under controlled conditions in 2013 and 2014. Liquid N applications significantly improved MEPP compared to granular N applications. Table 5.9 shows that spraying of plants with liquid N demonstrated a 24% advantage over soil applications in 2013. Although not statistically different, data showed that there was a better response from plants treated with liquid N compared to granular N in 2016. There is however, a noticeable poor response from these glasshouse grown plants in 2016 irrespective of the method of application. If the planting calender provided in Table 5.3 is taken into account, it could be speculated that environmental factors such as daylength or light intensity affected these responses although the noticeable differences in soil P status are more likely to be the causal effect of these differences as was mentioned above. A review by Liu et al. (2015) noted that low light intensity impaired net photosynthetic rate and lowered dry matter accumulation and sink capacity in rice, which in turn resulted in reduction in number of filled grains and 1000-grain weight (Sato 1956). Zhang et al. (2003) reported that root biomass, stems, leaves, photosynthetic rate, stomatal conductance and plant transpiration decreased under low light intensities, which could better explain these responses of the plants.

Table 5.9: Effect of method of N application on plant biomass per pot (PBPP), number of ears per pot (NEPP), mass of ears per pot (MEPP), grain yield per pot (GYPP) and harvest index (HI)

Year	Method	PBPP	NEPP	MEPP	GYPP	HI
2013	Soil	21.29b	7b	14.33b	10.43b	0.49
	Foliar	25.42a	8a	17.46a	12.35a	0.50
	Mean	**23.35**	**8**	**15.89**	**11.39**	**0.50**
	Cv (%)	**12.50**	**9.43**	**13.92**	**11.92**	**1.43**
	P value	***0.000***	***0.026***	***0.000***	***0.000***	***0.893***
2014	Soil	43.11	8	21.61b	15.77b	0.37
	Foliar	46.20	9	24.58a	17.13a	0.38
	Mean	**44.65**	**9**	**23.09**	**16.45**	**0.37**
	Cv (%)	**4.89**	**8.32**	**9.09**	**5.85**	**1.89**
	P value	***0.111***	***0.224***	***0.003***	***0.031***	***0.803***
2016	Soil	22.53	5	13.41	10.28	0.46b
	Foliar	20.52	5	13.40	11.10	0.53a
	Mean	**21.52**	**5**	**13.40**	**10.69**	**0.50**
	Cv (%)	**6.60**	**0.00**	**0.00**	**5.42**	**9.99**
	P value	***0.176***	***0.633***	***0.991***	***0.418***	***0.008***

Means within a column in each year differ significantly at $p = 0.05$ if they are followed by different letters

5.3.4 Grain yield per pot (GYPP)

The results of this study revealed that application of N fertiliser as UAN (60 kg N ha^{-1}) (14.95 g pot^{-1}), liquid urea (60 kg N ha^{-1}) (14.25 g pot^{-1}) and granular urea (60 kg N ha^{-1}) (12.67 g pot^{-1}) significantly ($p \leq 0.05$) increased mean GYPP compared to other N treatments in 2013 (Table 5.7). Plants that did not receive any N (control) produced the lowest mean grain yield (4.67 g pot^{-1}) in 2013. Nitrogen is the most limiting nutrient that affects plant growth and grain yield (Mandic et al. 2015). The responses demonstrated by these plants suggest that the method of application and N rate contributed significantly to the variations observed in grain yield. The responses tended to favour the liquid N applications compared to granular applications. Generally, the study showed that UAN 60 was consistent in improving grain yield under glasshouse conditions. Although there are concerns of foliar leaf scorch when liquid (foliar) applications exceed 30 kg N ha^{-1} (Poulton et al. 1990; Turley et al. 2001), there was no visible leaf scorch observed in this study both at 30 and 60 kg N ha^{-1}. This was probably due to the increased water carrier, which reduced the concentration thereby avoiding leaf damage.

N rate significantly affected the amount of grain produced per pot in all the years of the study (Table 5.8). Grain yield of wheat responded positively to increasing amount of N applied per pot. In 2013, plants top-dressed with N at 60 kg ha^{-1} produced 13.40 g pot^{-1} compared to 9.37 g pot^{-1} found in plants that received 30 kg N ha^{-1} as topdressing at tillering (Table 5.8). Similarly, in 2014, increasing N rate resulted into a 15% yield increase. Marino et al. (2009) and Noureldin et al. (2013) reported that increasing the N rate increased mean grain GY and yield components in wheat.

The effect of the method of N application showed that liquid N application performed significantly better compared to granular N applications in 2013 and 2014 as has been observed with other parameters above. A high mean grain yield (12.35 g pot^{-1}) was produced by plants that were sprayed with liquid N solutions compared to 10.43 g pot^{-1} produced by plants that received granular N in 2013 (Table 5.9). A similar trend was observed in the responses of grain yield in 2014. Khan et al. (2009) reported significant grain yield improvement amounting to 32% when 4% urea solution was applied at tillering compared to granular soil applied treatments in studies conducted in Pakistan. These findings are in agreement with those of Alston (1979) and Strong (1982), who reported increased grain yield influence from foliar (liquid) applied N. The differences in the response of grain yield found in this study could probably be due to the different mechanisms in which the N nutrient was absorbed and partitioned by these plants. Foliar applied nutrients are known to be taken up faster by plants compared to soil applied granular fertilisers (Fageria et al. 2009). Dawar et al. (2012) reported that approximately 30-40% N was recovered from foliar applied urea within few hours of application in pot experiments of perennial ryegrass. In contrast, Fageria et al. (2009) reported that crops respond to soil applied fertilisers in five to six days if climatic conditions are favourable. This study suggests that liquid N applications at tillering could offer a yield benefit over the conventional method of N application. However, several edaphic and climatic factors can contribute significantly to the availability, uptake and partitioning of N under field conditions making it difficult to provide conclusive recommendations on responses of grain yield to these fertilisers.

The interaction between the N rate and method of application in 2016 showed that the maximum mean grain yield was obtained with the application of N as a liquid N at 60 kg ha^{-1} (13.22 g pot^{-1}) (Figure 5.1). No significant differences were found between granular N at 30 kg ha^{-1} (10.29 g pot^{-1}), liquid N at 30 kg ha^{-1} (8.98 g pot^{-1}) and granular N at 60 kg ha^{-1} (10.27 g pot^{-1}). Penny et al. (1978) recorded significant increases in grain yield resulting from increased rate of liquid N compared to soil applied N sources.

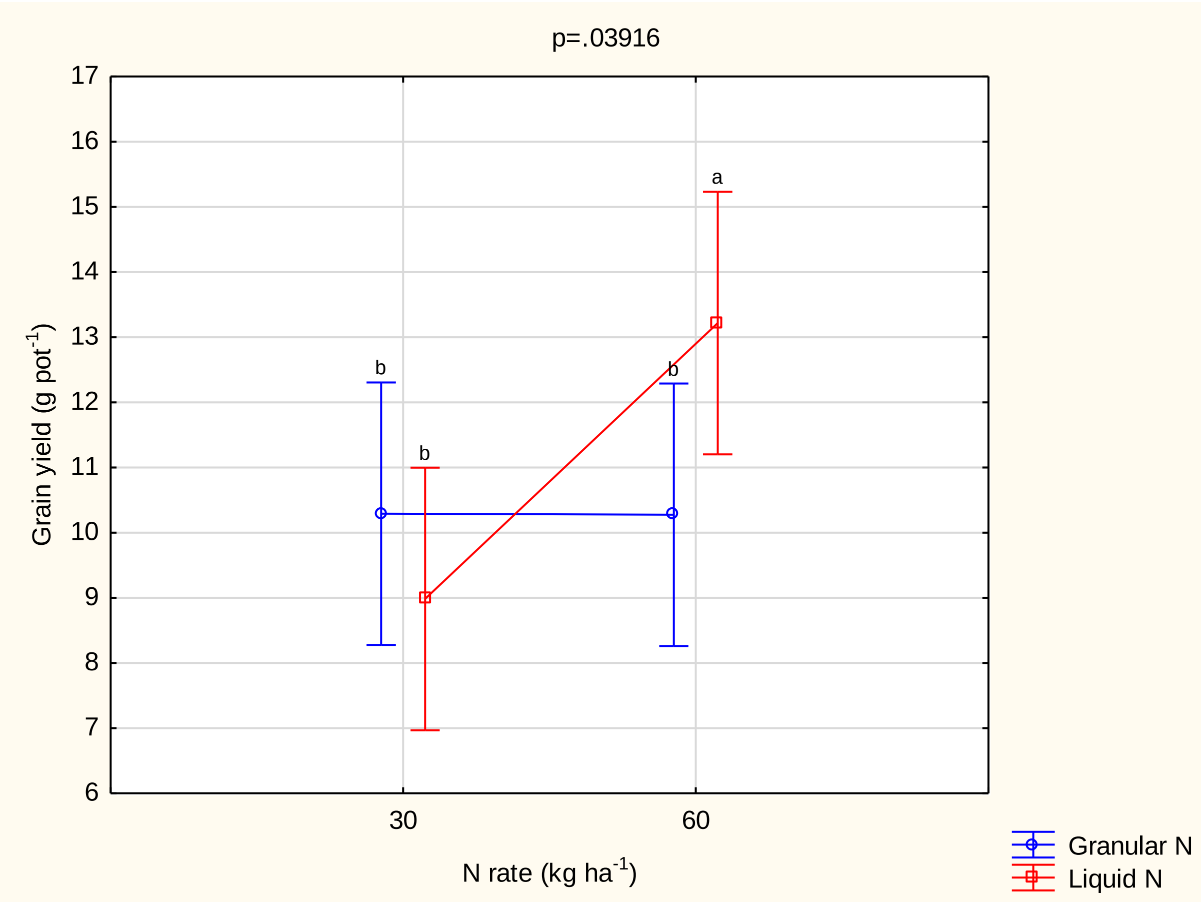

Figure 5.1: Effect of the interaction between nitrogen (N) application rate and method of application on grain yields of pot growth wheat plants (Different letters above bars indicate significant differences at p = 0.05)

5.3.5 Harvest index (HI)

The results of the study showed that HI was not affected by the N fertiliser treatment in 2013. The highest mean HI (0.65) was demonstrated by the control. Plants sprayed with liquid urea (60 kg N ha^{-1}) showed the lowest mean HI (0.46) (Table 5.7). Ahmad et al. (2015) reported that although grain filling, grain filling rate, 1000 gain weight and grain yield increased due to N rate; harvest index did not respond significantly in studies on the effect of N in source-sink relationship in wheat. The responses of HI, to a certain extent showed a positive relationship with grain yield. Plants that demonstrated higher grain yield also showed higher harvest indices. Fageria et al. (2011) reported a significant and quadratic relationship between grain yield and HI in lowland rice under glasshouse conditions. In 2014, UAN and solid urea, both at 60 kg N ha^{-1} level, produced the highest HI's. In 2016, UAN (60 kg N ha^{-1}) once again showed higher HI compared to other treatments.

The effect of the interaction between N rate and method of application showed that application of liquid N at 60 kg ha^{-1} produced the highest HI and was significantly different to other interactions (Figure 4.2). These results are similar to the observation of the N rate x method of application interaction on grain yield. As highlighted in the discussion on the effect of N treatment on HI above, there seemed to be a strong relationship between grain yield and HI in this study. An improvement in grain yield generally improved the HI. Studies by Amanullah et al. (2015) showed that foliar N applications significantly improved biological yield, grain yield and harvest index in studies on foliar N applications in wheat.

5.3.6 Grain protein content (GPC) and falling number (FN)

The results of this study showed that GPC was not significantly affected by the N topdressing treatments. The GPC values ranged between 7 and 8% as shown in Table 5.10. Compared to the responses found in field-conducted studies (Chapter 4), these plants showed poor GPC. This could be due to lack of sufficient N reserves during the critical period at post-anthesis and grain filling. Applications of N early in the growing season are generally known to favour grain yield more than GPC (Stark and Tindall 1992). Late N application is widely recognized in enhancing GPC (Wieser and Seilmeier 1998; Blandino et al. 2015; Xue et al. 2016). Delayed N benefits protein build-up over starch in the grain and assist in prolonging the duration of grain-filling (Sowers 1994).

The study revealed that the FN was not significantly affected by the N treatment (Table 5.10). Lack of significant responses from FN could be ascribed to the lower influence of N on this parameter in general as it is more associated with the growth conditions than N applications. Conner (1985) and Kettlewell (1996) reported that the environment, water availability and temperature after anthesis play a significant role on FN. Clarke et al. (2004) also reported inconsistent effect of N on FN where N increased FN in one season and resulted into a decrease of FN in the other season. The authors concluded that N could either increase or decrease FN.

Table 5.10: Summarised effect of N treatments on grain protein content (GPC) and Falling number (FN)

Treatment	GPC (%)	FN (s)
Control	8	318
LAN 30+	7	326
Granular LAN 30	8	329
Granular LAN 60	8	336
Granular Urea 30	8	353
Granular Urea 60	7	308
Liquid Urea 30	8	334
Liquid Urea 60	8	323
Liquid UAN 30	8	326
Liquid UAN 60	8	328
Mean	**8**	**328**
Cv (%)	**5.41**	**3.61**
P value	**0.224**	**0.789**

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate, + = LAN followed by foliar application of Activate N

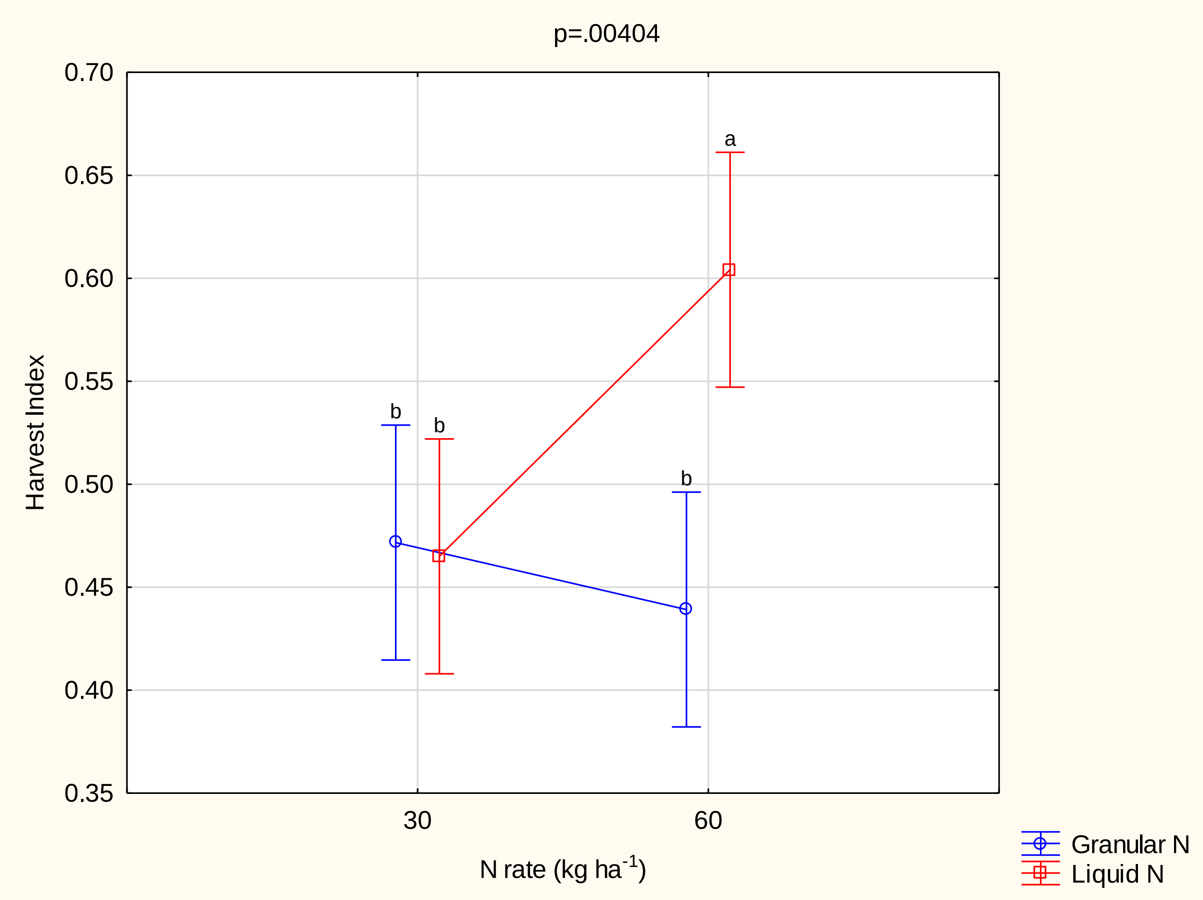

Figure 5.2: Effect of the interaction between nitrogen (N) application rate and method of application on harvest index of pot grown wheat plants (Different letters above bars indicate significant differences at p = 0.05)

5.3.7 Multiple linear regression analysis

The multiple linear regression analysis showed that plant biomass per pot and harvest index, which explained 93% of the variation, significantly affected grain yield in this study, (Adjusted R^2 = 0.93) (Table 5.11). Plant biomass showed the most significant effect, and the model indicates that a one unit increase in plant biomass will result into 116% increase in grain yield (b* = 1.16), provided that the harvest index is held constant. Similarly, the model showed that, if plant biomass is not increased, a one unit increase in harvest index would cause an increase of 60% in grain yield (b* = 0.59).

Table 5.11: Multiple linear regression analysis of plant biomass (PDM), harvest index (HI), number of ears (NE) and mass of ears (ME) on grain yield

	Regression Summary for Dependent Variable: Grain yield Multiple regressions (Grain yield as dependant variable) R= .96341822 R²= .92817467 Adjusted R²= .92736308 CV-R^2=0.92						
N=180	b*	Std.Err. of b*	b	Std.Err. of b	t(177)	p-value	# times in best 20 models
Intercept			-9.25301	0.584160	-15.8398	0.000000	
PDM	1.158816	0.024262	0.39372	0.008243	47.7636	0.000000	4
HI	0.596673	0.024262	22.49677	0.914748	24.5934	0.000000	4
NE	Excluded						4
ME	Excluded						4

5.4 Conclusions

The study revealed that application of UAN at 60 kg N ha^{-1} produced significantly better results than 30 kg N ha^{-1} and was consistent in all the years in terms of grain yield. The application rate of N significantly affected PBPP, NEPP, MEPP, GYPP and to a certain extent, the HI. The responses showed that 60 kg N ha^{-1} promoted positive and higher yields and yield parameters compared to 30 kg ha^{-1}. The method of application showed that liquid applied N was superior compared to granular applied N throughout the study. The effect of the interaction between N rate and method of application did not show any effect with the exception of GY and HI in 2016. The study showed that there was a strong relationship between HI and grain yield in two (2014 and 2016) of the three study years. The N fertiliser treatment did not have any significant effect on GPC and FN. The GPC values were generally low probably due to limited availability of N during the grain filling stage. The multiple linear regression analysis showed that plant biomass and harvest index significantly influenced grain yield. In conclusion, data obtained from these studies suggest that the application of N to the soil at sowing combined with liquid N topdressings at tillering can be beneficial to wheat in terms of yields and yield parameters.

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Chapter 6

Response of spring wheat (*Triticum aestivum* L) yield, grain quality and yield parameters to single and split applications of granular and liquid applied nitrogen under glasshouse conditions

Abstract

The effect of single and split granular and liquid applied nitrogen (N) on grain yield, grain quality and yield parameters of spring wheat (*Triticum aestivum* L.) were evaluated under glasshouse conditions in 2014, 2015 and 2016. The randomised complete block design experiment with 13 treatments was replicated five times. The treatments consisted of soil applied Urea (46%), Urea (46%) solution and Urea Ammonium Nitrate (32%) and these were applied either once at tillering or twice at tillering and flowering respectively. All treatments except the control received Limestone Ammonium Nitrate (LAN 28%) at planting at amounts equivalent to 30 kg N ha^{-1}. A subset of the data was analysed as a 2x2x2 factorial analysis to investigate the effects of rate of application, method of application and timing of application and their possible interactions. The control treatment did not receive any nitrogen. Nitrogen treatments significantly ($p \leq 0.05$) affected plant biomass, number of ears, mass of ears, grain yield and harvest index in 2014 and 2016. Plant responses increased with increasing N rates. The method x timing interaction showed significant differences due to timing of N application for liquid applied N sprays. Plants treated with liquid N once at tillering showed superior responses compared to split applications of liquid N. The multiple linear regression analysis showed that mass of ears and to a lesser extent, the harvest index significantly influenced grain yield. Grain protein content and falling number did not show any significant responses to N treatments.

Keywords: Granular N, grain protein content, liquid N , UAN solution, urea solution

6.1 Introduction

Nitrogen (N) plays a critical role in achieving optimum crop grain yield (Ooro et al. 2011) and is considered one of the most common nutrients limiting yield of wheat and other cereal crops (Moll et al. 1982; Blumenthal et al. 2008). Its application is usually the largest production cost in most cereal grain crops (Schwenke et al. 2014). Nitrogen use efficiency (NUE) is currently between 40 and 50% for most cereal production systems (Gupta and Khosla 2012) and this is slightly higher than the 33% previously mentioned by Raun and Johnson (1999). Moll et al. (1982) reported that plant NUE is affected by several factors including application time, rate of applied N, cultivar and climatic conditions. Use of foliar applications of N in efforts to improve grain yield and quality of wheat has shown a great potential although the results remain inconsistent (Fageria et al. 2009). The general assessment suggests that responses to liquid N spray applications have not been consistent, some research results have been positive; others negative, while no responses were recorded for some depending on crop species and nutrient applied (Fageria et al. 2011).

According to Kinaci and Gulmezoglu (2007), time of nutrient application plays a significant role in improving the efficiency of the foliar treatment. Applying the fertilizer in three or more doses supports certain yield components depending on the time of application and the corresponding stage of plant development (Alcoz et al. 1993). Furthermore, a split application reduces the risk of lodging and N losses by leaching (Gerwing et al. 1979; Kanwar et al. 1988, Varshney et al. 1993) because of the balance between N application and N uptake in both time and the amount. Arregui and Quamada (2008) reported no significant decrease or increase in yield, biomass or grain N when a single dose was applied compared with split doses. Chen et al. (2016) found that split application of N significantly improved grain yield at high N rates but not at low N rates compared to a single banded application in studies conducted in China.

Applying N strategically in response to crop growth and challenges experienced during crop development (e.g. poor rooting or dry soil conditions) could be more efficient in crop nutrition. Liquid (foliar) N would be a special tool under such strategies (Turley et al. 2001). Liquid sprays can be used strategically to manage crop canopies when soil moisture is limiting during the growing season or when N uptake by roots is restricted. Liquid application of nutrients is highly beneficial because crops can benefit when roots are unable acquire the nutrients at a critical stage (Brar and Brar 2004). Furthermore, foliar N may be beneficial under conditions where rooting is limited and N uptake affected by diseases such as take-all (*Gaemannomyces graminis*) (Turley et al. 2001).

Advances in agriculture include reducing the cost of production, maintaining soil quality and potential increase of agro-ecosystems for human and animal health. The increase in use of liquid foliar sprays is motivated by the development of high concentration of soluble fertilisers and improvement in use of machinery for spraying fungicides, herbicides and insecticides, and irrigation systems facilitates the application of nutrients to crops in the form of sprays (Fageria et al. 2009). Studies incorporating foliar applications of N fertilizer sources in wheat production systems are generally limited in South Africa. The objective of this study is to evaluate the response of spring wheat to different granular and liquid applied N fertilisers when applied as a single dose (at tillering) and split between tillering and flowering.

6.2 Materials and methods

An experiment was conducted under glasshouse conditions for three seasons (2014-2016) at Welgevallen Experimental Farm, Department of Agronomy, University of Stellenbosch, Stellenbosch, South Africa (33°56'33"S, 18°51'56"E, 136 m.a.s.l.). The experiment was designed as a Randomized Complete Block Design with 13 treatments replicated five times. Wheat cultivar SST 027 was planted in pots filled with field soil as a growing medium. The area of the pots was 0.02 m^2. About 0.21 g pot^{-1} of Limestone Ammonium Nitrate (LAN 28% N) was applied in all the pots at sowing with the exception of the control (0 N). The allocation of treatments is shown in Table 6.1. The chemical properties of the soil used as a growing medium is shown in Table 6.2. After planting, the growing medium was kept moist using tap water. After emergence, pots were irrigated manually to field capacity with a nitrogen free balanced standard nutrient solution (Table 6.3) every three to four days depending on the moisture status of the soil. The glasshouse was set at a 10/25°C night/day temperature throughout the study period.

Two weeks after emergence, plants were thinned from five plants to three plants pot^{-1}. To evaluate the effect of N timing, some of the treatments were applied once at tillering (Zadoks GS 21), while others were applied both at tillering and flowering (Zadoks GS 61) (Zadoks et al. 1974). Table 6.1 shows the treatments applied to the wheat. For liquid urea ammonium nitrate (UAN) (32%) treatment, the solution was prepared by mixing 44.38, 88.75 and 177.5 ml of UAN solution with 500 ml of water. The lowest N rate (44.38 ml 500 ml^{-1}) was applied at tillering growth stage and repeated again at early flowering stage. The 88.75 ml 500 ml^{-1} N rate was applied once at tillering and twice at tillering and early flowering. The highest N level (177.5 ml. 500 ml^{-1}) was applied once at tillering. To prepare the liquid urea solution, 40.75, 81.5 and 163 g of urea granules were dissolved in 500 ml of water. The sequence of application followed the description given above for UAN. The liquid N treatments were applied during the morning hours of the day using a pot-spraying apparatus at a pressure of 2 kPa and with a delivery rate of 400 litres of water ha^{-1} The liquid sprays were applied on top of the plant material, which means that a certain amount was intercepted by the leaf material while the rest fell on the soil surface. The N solutions were prepared to apply N amounts equivalent to those applied as granular urea. The granularurea treatments were applied by spreading urea granules on top of the soil surface of the pot using 0.07, 0.13, and 0.26 g of urea per pot and the three N rates were applied according to the description of the sequence given above. The application rates were selected to give N levels of approximately equal to 15, 30 and 60 kg N ha^{-1} on an area basis. The order of activities conducted in the experiment is shown in

Table 6.4. The planting dates in the glasshouse depended largely on the availability of working space, which explains the different times of planting in each year.

At physiological maturity, above ground plant material was cut off at the stem base and samples of each treatment were collected, dried until constant weight (55°C for 72 hours), weighed and threshed. Plant biomass pot^{-1} (PBPP), number of ears pot^{-1} (NEPP), mass of ears pot^{-1}(MEPP), grain yields pot^{-1} (GYPP) were measured and the harvest index (HI) was calculated by dividing the total biomass (above ground) by the total grain yield. Grain protein content was determined at the Welgevallen Experimental Farm at the University of Stellenbosch using the Near-Infrared Reflectance method for protein in wheat flour AACC Method 39-11 (American Association of Cereal Chemists 2000). The falling numbers were determined following the standard procedure according to the American Association of Cereal Chemists (2000) at the Welgevallen Experimental Farm.

Table 6.1: Nitrogen topdressing treatments in the glasshouse experiment in 2014, 2015 and 2016

Treatment	**Method**	**Sowing (LAN) (g pot^{-1})**	**Tillering**	**Flowering**
Control	0	0	0	0
Urea 30 Single	Granular	0.21	0.13 gpot^{-1}	0
Urea 30 Split	Granular	0.21	0.07 g pot^{-1}	0.07 g pot^{-1}
Urea 60 Single	Granular	0.21	0.26 g pot^{-1}	0
Urea 60 Split	Granular	0.21	0.13 g pot^{-1}	0.13 g pot^{-1}
Urea 30 Single	Liquid	0.21	81.5 g 500 ml^{-1}	0
Urea 30 Split	Liquid	0.21	40.75 g 500 ml^{-1}	40.75 g 500 ml^{-1}
Urea 60 Single	Liquid	0.21	163 g 500 ml^{-1}	0
Urea 60 Split	Liquid	0.21	81.5 g 500 ml^{-1}	81.5 g 500 ml^{-1}
UAN 30 Single	Liquid	0.21	88.75 ml 500 ml^{-1}	0
UAN 30 Split	Liquid	0.21	44.38 ml 500 ml^{-1}	44.38 ml 500 ml^{-1}
UAN 60 Single	Liquid	0.21	177.5 ml 500 ml^{-1}	0
UAN 60 Split	Liquid	0.21	88.75 ml 500 ml^{-1}	88.75 ml 500 ml^{-1}

UAN = Urea ammonium nitrate

A one-way analysis of variance (ANOVA) was performed to test for differences between treatments for all parameters using the GLM (General Linear Model) Procedure of the

Statistica, Version 13.2 (StatSoft, Inc., Tulsa, OK, USA). Means were separated using the Fisher's protected least significant difference (LSD) test at $p = 0.05$. In cases where residuals were not normally distributed, the Kruskal-Wallis test was used as a non-parametric test to confirm the results of the ANOVA. In cases where Levene's test for homogeneity of variances indicated heterogeneous variances, the LSD test was replaced with the Games-Howell multiple comparison procedure.

For the analyses of N timing, N rate, N method and their interactions, data sets from granular and liquid N applied once at tillering or twice between tillering and early anthesis were extracted. This data was analysed as a 2 (two methods of application: granular, liquid) x 2 (two N rates: 30 and 60 kg N ha^{-1}) x 2 [timing of application: tillering (T) and tillering and flowering (TF)] factorial. The N rate analyses considered the total amount of N topdressing irrespective of the time of application. This means that the two applications of N at 15 kg ha^{-1} were analysed as 30 kg N ha^{-1} topdressing rate together with a single topdressing of N at 30 kg ha^{-1}. The single application of N at 60 kg ha^{-1} and the split applications of N at 30 kg ha^{-1} between tillering and early anthesis accounted for 60 kg ha^{-1} N rate. The variance estimation, precision and comparison (VEPAC) package of Statistica was used for statistical analyses. To analyse the grain protein content, the grain samples from 2014 and 2016 were used as replicates. One of the challenges was that the sample per treatment was too small to meet the minimum requirements for the analysis and therefore, samples from the five replicates per treatment were combined to represent the treatment. In 2015, the grain yield was reduced severely due to a fungal disease infection and as such, the grain protein content analyses were omitted.

Multiple linear regression analysis was performed for all the tested variables and grain yield was the dependant variable to assess which variable/s contributed significantly to the grain yield and to further evaluate the relationship between the different variables. Where there was severe multi-collinearity among the input variables, the best five input variables were selected using the best subsets procedure and variables were then reduced to the best three or two input variables.

Table 6.2: Soil characteristics of the Welgevallen soils used in the study for 2014, 2015 and 2016

	pH (KCl)	Resist.	H^+	P (Bray II)	K	Na	K	Ca	Mg	Cu	Zn	Mn	B	C	Total N	S
Year	KCl	Ohm	cmol.kg^{-1}	mg kg^{-1}	mg kg^{-1}	cmol(+).kg^{-1}				mg kg^{-1}				(%)		mg kg^{-1}
2014	4.7	1120	0.86	126	91	0.05	0.23	1.89	0.33	2.2	3.4	41.5	0.1	0.87	0.06	13.31
2015	4.9	1280	0.68	90	111	0.04	0.25	3.08	0.51	1.9	3.58	50.37	0.24	0.8	0.05	7.8
2016	6.1	2270	0.63	59	87	0.08	0.22	3.39	0.67	2.9	4.4	132	0.28	0.61	0.06	12.44

Table 6.3: The chemical composition of the nutrient solution applied on wheat plants in the glasshouse in 2014, 2015, 2016

pH	EC	Na	K	Ca	Mg	Fe	Cl	CO	HCO	SO	B	Mn	Cu	Zn	P	NH-N	NO-N
KCl	mS.m^{-1}	mg.l^{-1}															
6.1	147.3	14.7	280.5	160	52.2	1.46	25.6	0	57	693	0.52	0.16	0.11	0.18	82.6	0.47	6.13

Table 6.4: The dates of different activities in the glasshouse from 2014 - 2016

Sowing	N 1 - Tillering	N 2 - Flowering	Harvesting
15 Oct. 2014	16 Nov. 2014	08 Jan. 2015	12 Feb. 2015
29 Aug. 2015	02 Oct. 2015	03 Nov. 2015	23 Dec. 2015
25 Apr. 2016	20 May 2016	27 Jul. 2016	27 Sep. 2016

6.3 Results and discussions

The summary of the Anova analysis is shown in Table 6.5. The plant biomass, number of ears, mass of ears, grain yield and harvest index were significantly ($p \leq 0.05$) affected by the treatments in 2014 and 2016. In 2015, only plant biomass and number of ears showed significant responses to treatment effect.

The N application rate significantly influenced plant biomass, number of ears, mass of ears and grain yield in 2014 and 2016 (Table 6.6). In 2015, this effect was only significant for plant biomass and number of ears. The study showed that there were almost negligible responses in wheat plants to the effect of method of N application. Only plant biomass, mass of ears and grain yield were significantly affected by the method of application during the 2016 season. The timing of N application did not show any significant effect to the studied parameters. The interaction between N rate and timing of N application did not significantly influence wheat growth and development. The only parameter that responded to this interaction is the HI in 2016. The interaction between the method of N application and timing affected responses of spring wheat in the glasshouse in 2014. No significant effect was shown by the interactions between N rate, method of application and timing.

The fertilizer N treatment did not influence the grain protein content and falling number (Anova not shown). The summary of the effects of N treatment on GPC and FN is shown in Table 6.9.

Table 6.5: The summary of the Anova analysis for the effect of N treatment (NT) on plant biomass, number of heads, mass of heads, grain yield and harvest index in 2014, 2015 and 2016

	N treatment		
Variable	**2014**	**2015**	**2016**
Plant biomass	*	*	*
No. of ears	*	*	*
Mass of ears	*	ns	*
Grain yield	*	ns	*
Harvest index	*	ns	*

*****Significant at $p = 0.05$, ns – not significant at $p = 0.05$

Table 6.6: The summary of the Anova analysis for the effect of N rate (R), method of N application, (M), timing (T), N rate x timing, method x timing, and N rate x method x timing interaction of plant biomass, number of heads, mass of ears, grain yield and harvest index in 2014, 2015 and 2016

		Source					
Year	**Variable**	**R**	**M**	**T**	**R x T**	**M x T**	**R x M x T**
2014	Plant biomass	*	ns	ns	ns	*	ns
	No. of ears	*	ns	ns	ns	*	ns
	Mass of ears	*	ns	ns	ns	*	ns
	Grain yield	*	ns	ns	ns	*	ns
	Harvest index	ns	ns	ns	ns	*	ns
2015	Plant biomass	ns	ns	ns	ns	ns	ns
	No. of ears	ns	ns	ns	ns	ns	ns
	Mass of ears	ns	ns	ns	ns	ns	ns
	Grain yield	*	ns	ns	ns	ns	ns
	Harvest index	*	ns	ns	ns	ns	ns
2016	Plant biomass	*	*	ns	ns	ns	ns
	No. of ears	*	ns	ns	ns	ns	ns
	Mass of ears	*	*	ns	ns	ns	ns
	Grain yield	*	*	ns	ns	ns	ns
	Harvest index	*	ns	ns	*	ns	ns

*****Significant at $p = 0.05$, ns – not significant at $p = 0.05$

6.3.1 Plant biomass per pot (PBPP)

As indicated in Table 6.6 above, plant biomass showed significant responses to the N treatments ($p \leq 0.05$). In 2014, the highest mean plant biomass (34.6 g pot^{-1}) was produced by liquid urea applied at 60 kg N ha^{-1} although the response was not significantly different to a number of other treatments (Table 6.7). The responses were rather complex probably due to the effect of N rate, method and timing of application to plant growth and development. The control (0 N) treatment, as expected produced the lowest mean plant biomass (20.10 g pot^{-1}). In 2015, there was a noticeable decline in the production of plant dry matter compared to the 2014 responses. This trend was also observed for other tested parameters. This was probably due to stunted growth (visual observation) which resulted following the infection of the plants by a fungal leaf disease at early growth stages (2 weeks after germination) in 2015. Although these plants were treated with a fungicide, they did not recover fully from the negative effect of the disease infection. The infection influenced plant growth and development and this appears to have reduced the total plant biomass produced per pot in 2015. Herrera-Foessel et al. (2006) mentioned that plants respond to disease inoculation with energy-demanding physiological processes, probably defense reactions, using stored host energy that otherwise would go to growth and seed production. In addition to this, Samborski and Peturson (1960) stated that, a reduction in photosynthetic leaf area also could cause yield reductions, explaining the low responses in 2015. Although these differences could somewhat be associated with fungal infection (2015) or different time of planting (2015), the higher soil P levels in the growing medium could be the main reason of improved plant biomass in 2014 compared to other years.

The maximum plant biomass was produced by the urea liquid (60 kg N ha^{-1}) treatment again in 2015 and the control produced significantly lower plant biomass (Table 6.7). The positive responses shown by these plants to liquid urea applications could be ascribed to the high solubility of urea thereby enabling a rapid uptake of the applied N. According to Bowman (1992), urea is the most common source of foliar N applications because it is highly soluble and has relatively low potential for injuring foliage. Wittwer et al. (1963) reported that urea is absorbed faster by leaves than either NO_3^- or NH_4^+, because nonpolar substances including urea diffuse through the cuticle more easily. Klein and Weinbaum (1984) also stated that, urea is absorbed, metabolized and translocated very rapidly after application. Less significant differences were observed between treatments in 2016. The plant biomass varied between 16.80 and 11.50 g pot^{-1} from urea solid (60 kg N ha^{-1}) and UAN (30 kg N ha^{-1}) respectively.

The effect of N rate on plant biomass is illustrated in Table 6.8. Increasing the amount of N applied to the plants significantly improved the production of dry plant material by the pot-grown wheat plants in 2014 and 2016. The responses of plant dry matter ranged between 31.35 and 28.35 g pot^{-1}in 2014. Li et al. (2016) reported that increasing N rate significantly increased dry matter production up to 150 kg N ha^{-1} in wheat studies conducted in China. In 2016, top-dressing wheat plants with 60 kg N ha^{-1} significantly improved plant growth and development. The responses favouring higher N rates could be due to increase in photosynthetic rate and higher leaf area resulting into increased total dry matter production

(Rahman et al. 2014). These results are in agreement with the findings of Kumar and Sharma (1999), who reported that dry matter production in wheat increased significantly when N rate was increased.

The effect of the method of N application on PBPP in 2016 showed that granular N differed significantly to the liquid applied N and PBPP varied from 14.96 g pot^{-1} for granular N application to 13.10 g pot^{-1} for liquid N application (Data not shown). Granular N applications probably encouraged better plant N uptake, which enhanced plant biomass production observed at maturity. The pathways followed by plants in the uptake of N from the soil and via the leaf are different. Although many studies reported significant improvement of crop yields and yield components through foliar N applications Fageria et al. (2009) mentioned that the responses to foliar N applications are not consistent. It could be speculated that liquid N applications resulted into poor N uptake and recovery due to N volatilization (Below et al. 1985) compared to granular applied N. Studies by Mbangcolo and Pieterse (2017) under similar glasshouse conditions also showed superior dry matter production from granular soil applied N compared to liquid foliar applied N in measurements taken at three different growth stages (stem extension, early heading and physiological maturity).

Table 6.7: Effect of N treatment on plant biomass per pot (PBPP), number of ears per pot (NEPP), mass of ears per pot (MEPP), grain yield per pot (GYPP) and harvest index (HI) in 2014, 2015 and 2016 under glasshouse conditions

Year	N treatment	PBPP	NEPP	MEPP	GYPP	HI
		g		g		
2014	Control	20.10d	3c	6.60e	4.70d	0.24c
	Granular Urea 30T	28.50bc	5ab	12.30bcd	8.98bc	0.32ab
	Granular Urea 15TF	29.90abc	6ab	13.60abc	10.20abc	0.33ab
	Granular Urea 60T	30.30ab	5ab	13.10bcd	9.70abc	0.31b
	Granular Urea 30TF	31.40ab	7a	14.70ab	11.60a	0.37a
	Liquid Urea 30T	30.32ab	5ab	13.40abcd	10.00abc	0.33ab
	Liquid Urea 15TF	27.40bc	4bc	11.40cd	8.60bc	0.31ab
	Liquid Urea 60T	34.60a	7a	16.30a	11.80a	0.34ab
	Liquid Urea 30TF	30.70ab	6ab	14.20abc	10.40abc	0.34ab
	Liquid UAN 30T	27.20bc	5ab	13.00bcd	10.00abc	0.37a
	Liquid UAN 15TF	25.10bcd	3c	10.40d	7.90c	0.31b
	Liquid UAN 60T	33.30ab	7a	15.20ab	11.40a	0.34ab
	Liquid UAN 30TF	28.80abc	5ab	14.10abc	9.90abc	0.34ab
	Mean	**29.05**	**5.31**	**12.95**	**9.63**	**0.33**
	Cv (%)	**12.70**	**26.07**	**18.99**	**19.37**	**10.02**
	P Value	***0.001***	***0.000***	***0.000***	***0.000***	***0.001***
2015	Control	4.68e	3	4.07d	3.15d	0.68
	Granular Urea 30T	7.74abcd	3	6.00abc	3.84bcd	0.50
	Granular Urea 15TF	8.42abc	3	6.64a	4.80abc	0.58
	Granular Urea 60T	8.39abc	3	6.85a	5.80a	0.70
	Granular Urea 30TF	8.72ab	4	6.93a	5.42a	0.62
	Liquid Urea 30T	8.67ab	3	6.55ab	5.20ab	0.60
	Liquid Urea 15TF	7.50abc	3	6.04abc	4.60abcd	0.61
	Liquid Urea 60T	8.81a	3	7.06a	5.20ab	0.60
	Liquid Urea 30TF	7.35abcd	3	6.05abc	4.68abc	0.65
	Liquid UAN 30T	6.71cd	3	4.98bcd	3.60cd	0.52
	Liquid UAN 15TF	6.77bcd	3	5.88abc	4.97abc	0.74
	Liquid UAN 60T	5.84de	3	4.85cd	3.72bcd	0.63
	Liquid UAN 30TF	8.17abc	3	5.98abc	5.20ab	0.63
	Mean	**7.52**	**3.12**	**5.99**	**4.63**	**0.62**
	Cv (%)	**16.55**	**9.01**	**14.88**	**17.44**	**10.66**
	P Value	***0.029***	***0.310***	***0.020***	***0.050***	***0.072***

Values in a column differ significantly at $p = 0.05$ if they are followed by different letters

UAN = Urea ammonium nitrate (32% N), T = applied tillering, TF = applied at tillering and Flowering

Table 6.7 Continued from the previous page

Year		PBPP	NEPP	MEPP	GYPP	HI
		g		g		
2016	Control	13.20b	3	8.70ab	6.04bc	0.47ab
	Granular Urea 30T	12.06b	3	8.24ab	6.70abc	0.50ab
	Granular Urea 15TF	14.34ab	3	8.70ab	6.78abc	0.47ab
	Granular Urea 60T	16.80a	4	10.38a	8.06a	0.44abc
	Granular Urea 30TF	16.64a	3	10.50a	7.98a	0.47ab
	Liquid Urea 30T	12.36b	3	8.10ab	5.76bcd	0.47ab
	Liquid Urea 15TF	12.04b	3	8.44ab	4.20d	0.35c
	Liquid Urea 60T	14.60ab	3	8.54ab	6.06bc	0.43abc
	Liquid Urea 30TF	11.74b	3	7.62b	5.70bcd	0.49ab
	Liquid UAN 30T	11.50b	3	7.60b	6.08bc	0.53a
	Liquid UAN 15TF	14.10ab	3	8.80ab	5.40cd	0.39bc
	Liquid UAN 60T	13.94ab	3	9.04ab	6.38abc	0.45abc
	Liquid UAN 30TF	14.52ab	3	9.70ab	7.38ab	0.51a
	Mean	**13.68**	**3.2**	**8.8**	**6.35**	**0.46**
	Cv (%)	**14.49**	**9.01**	**10.43**	**16.71**	**10.65**
	P Value	***0.002***	***0.320***	***0.000***	***0.030***	***0.022***

Values in a column differ significantly at $p = 0.05$ if they are followed by different letters

UAN = Urea ammonium nitrate (32% N), T = applied at tillering, TF = applied at tillering and flowering

According to Mehrotra and Singh (1982), higher doses of N fertilization result into increased dry matter production over lower doses of N in wheat. The responses in 2015 were generally lower compared to those observed in 2014. As indicated above, a leaf disease that affected the plants at a very early growth stage (2 weeks after emergence) resulted into stunted growth. It can also be speculated that differences in the time of planting over the years probably resulted into variations in growth and development responses, consequently yield, and yield parameters.

Table 6.8: Effect of N rate on plant biomass per pot (PBPP), number of ears per pot (NEPP), mass of ears per pot (MEPP), grain yield per pot (GYPP) and harvest index (HI) in 2014, 2015 and 2016

Year	N rate (kg.ha^{-1})	PBPP (g)	NEPP	MEPP (g)	GYPP (g)	HI
2014	30	28.35a	5a	12.5a	9.36a	0.32
	60	31.35b	6b	14.43b	10.76b	0.34
	Mean	**29.85**	**6**	**13.47**	**10.06**	**0.33**
	Cv (%)	**7.1**	**12.86**	**10.13**	**9.84**	**4.29**
	P Value	***0.002***	***0.006***	***0.007***	***0.009***	***0.350***
2015	30	7.75	3	6.09	4.46a	0.58a
	60	8.05	3	6.44	5.16b	0.64b
	Mean	**7.9**	**3**	**6.27**	**4.81**	**0.61**
	Cv (%)	**2.69**	**0.00**	**3.95**	**10.29**	**6.96**
	P Value	***0.527***	***0.144***	***0.377***	***0.047***	***0.045***
2016	30	12.85a	3	8.35a	6.05a	0.46
	60	15.21b	3	9.58b	7.2b	0.46
	Mean	**14.03**	**3**	**8.97**	**6.63**	**0.46**
	Cv (%)	**11.89**	**0.00**	**9.7**	**12.27**	**0.00**
	P Value	***0.001***	***0.066***	***0.024***	***0.007***	***0.913***

Values in a column differ significantly at $p = 0.05$ if they are followed by different letters

6.3.2 Number of ears per pot (NEPP)

The results of this study showed that the NEPP was significantly ($p \leq 0.05$) affected by N treatment in 2014. The highest mean NEPP was produced by plants that received split applied granular urea (30 kg N ha^{-1}), liquid urea (60 kg N ha^{-1}) and UAN (60 kg N ha^{-1}) (Table 6.7). However, these three treatments were not statistically different to several other treatments with the exception of the control and the split applied liquid N (15 kg N ha^{-1}). With the exception of granular urea (30 kg N ha^{-1}), the responses were favoured by an increase in N rates (Table 6.7), illustrating the role of nitrogen in the production of plant ears (spikes). Nakano et al. (2008) reported that increasing N rate increased the number of spikes per m^2 when N was applied at tillering in studies of nitrogen rate and timing in Southwestern Japan. This response could be attributed to the adequate N availability, which probably facilitated tillering ability (Jan and Khan 2000) and eventually higher number of ears per pot. Ayoub et al. (1994) reported that spike population per unit area increased with increasing N level in studies conducted on spring wheat in Canada. Zebart and Sheard (1992) reported that applications of 130 kg N ha^{-}

^{1} increased N consumption, producing 360 ears per unit area compared to 314 ears found with 50 kg N ha^{-1} on studies in winter wheat in Canada. No significant treatment effect was observed for NEPP in 2015 and 2016. The NEPP varied between three and four ears pot^{-1} for both years.

The interaction between the method of application and timing showed that there were no significant differences between split granular applied N and the liquid N applications at tillering (Table 6.9). Split applications of granular N application and a single application of liquid N (tillering) triggered improved ear formation compared to the single application of granular N and splitting of liquid N. These results demonstrate two contrasting responses from these pot-grown plants. Single applications of liquid N at early tillering encouraged the formation of extra ears, which improves grain yield compared to the splitting of the application. On the one hand, for granular soil applied N, splitting the fertilizer was more effective than a single application. With respect to liquid applied N, Mossedaq and Smith (1994) reported that N application just prior to stem elongation increased wheat yield compared to applications at anthesis or in equal parts at both stages due to kernel number per unit area. Applications of liquid N at tillering probably increased the N uptake efficiency, translocation and mobilization, which improved the number of ears produced per pot. In contrast, Papakosta and Gagianas (1991) reported that the beneficial effect of splitting soil applied N result into the reduction of N losses and to a greater translocation of pre-anthesis assimilates to the grain. This statement is in agreement with the responses observed for the interactive effect of method and timing of application for granular applied N. The reaction demonstrated by these plants suggests that the mechanism through which N from granular and liquid applied N sources is absorbed, assimilated and translocated into different growth components may be different.

6.3.3 Mass of ears per pot (MEPP)

This study revealed that MEPP showed significant (*$p \leq 0.05$*) responses to N treatments in all the years of the study except 2015 (Table 6.5). In 2014, liquid urea (60 kg N ha^{-1}) produced the highest mass of ears (16.30 g pot^{-1}) although the effect did not differ significantly with several other treatments as shown in Table 6.7. The poor ear mass responses were found in plants that did not receive any N as expected. These results highlight the importance of N in improving several wheat yield parameters including mass of ears. Veesar et al. (2017) reported that N availability at critical stages of wheat growth and development is of paramount importance. Chaudhry and Mehmood (1998) reported that N fertilizer had a significant effect on spike grain weight and consequently spike weight in studies conducted in two wheat varieties in Faisalabad. In 2016, data showed that there were less significant effects between treatments compared to the other years. The MEPP varied between 10.50 g (split granular urea at 30 kg N ha^{-1}) and 7.6 g (UAN at 30 kg N ha^{-1}).

The effect of N rate on mass of ears per pot was significant (*p≤0.05*) in 2014 and 2016. Table 6.8 shows that increasing the rate of N significantly improved the mass of ears produced by wheat plants in these two years. The highest mean mass of ears produced in 2014 was 14.43 g pot^{-1} (60 kg N ha^{-1}) while the lowest was 12.5 g pot^{-1} (30 kg N ha^{-1}). These results are in conformity with results obtained by Hussain et al. (2006) who reported that mass of ears increased as amount of N was increased from 0 N (control) to 200 kg ha^{-1}. The authors found that the highest ear mass (3.5 g plant^{-1}) was produced when N dose was 200 kg ha^{-1} while the lowest ear mass (2.8 g plant^{-1}) was recorded in the control. This is lower than the 4.81 g plant^{-1} (90 kg N ha^{-1}) and 4.16 g plant^{-1} (60 kg N ha^{-1}) of this study during 2014. No significant responses were observed in 2015.

The method x timing interaction significantly *(p≤0.05*) affected the mass of ears produced in 2014. Table 6.9 shows that no statistical differences were found between both granular N applications and the liquid N at tillering application. However, significant lower responses were recorded for split applications of liquid applied N. The trend shows that a slight advantage was gained when N was applied at tillering using the liquid method of application. There is a clear indication that the method x timing interaction influenced the mass of ears and the effect was more strongly associated with the timing of N application than the method. According to Alcoz et al. (1993), splitting efficiency of N depends on weather conditions influencing N losses and consequently N fertilisation efficiency. Chen et al. (2016) reported that the rational strategy for applying N to dryland cereals depends on the interaction between soil N, rainfall distribution and N uptake over time. Since the study was conducted under glasshouse conditions, the variations in responses could probably be due to soil N and N uptake efficiency.

6.3.4 Grain yield per pot (GYPP)

The results on the effect of N treatment on grain yields are shown in Table 6.6. In 2014, the highest mean GYPP (11.8 g) was obtained in plants that were sprayed with urea (60 kg N ha^{-1}) at tillering although this did not differ significantly from other treatments. The plants that did not receive any N fertiliser demonstrated poor growth and as such produced the lowest mean GY compared to other plants. Emam and Borjian (2000) reported yield increases resulting from improved number of grains ear^{-1} in studies on foliar urea applications in Iran, highlighting the role of different yield components on final grain yield. In 2015, the results showed that the highest mean GY (5.8 g pot^{-1}) was produced by plants that were treated with a single application of solid urea (60 kg N ha^{-1}) but this did not differ statistically to other treatments.

The control treatment showed the lowest GY responses compared from other treatments. In 2016, GYPP varied from 8.06 g pot^{-1} (granular urea at 60 kg N ha^{-1}) to 4.2 g pot^{-1} (split liquid urea at 15 kg N ha^{-1}). Spraying wheat plants with liquid urea (15 kg N ha^{-1}) at tillering and flowering growth stage showed negative responses suggesting that these wheat plants failed to respond to sprayed N at low levels. Bly and Woodard (2003) reported that foliar application of N at boot stage significantly reduced grain yield (5%) in studies conducted on hard red spring wheat cultivars in South Dakota, USA.

Table 6.9: The effect of the interaction between method (M) and time (T) of N application on PBPP, NEPP, MEPP, GYPP and HI in 2014, 2015 and 2016

Year	M x T	PBPP (g)	NEPP	MEPP (g)	GYPP (g)	HI
2014	G x T	29.40ab	5b	12.70ab	9.34ab	0.31a
	G x TF	30.65a	6a	14.15ab	10.90a	0.35ab
	L x T	31.35a	6a	14.48a	10.80a	0.34ab
	L x TF	28.00b	5b	12.53b	9.20b	0.33ab
	Mean	**29.85**	**6.00**	**13.46**	**10.06**	**0.33**
	Cv (%)	**4.94**	**8.70**	**7.38**	**9.10**	**5.14**
	P Value	***0.017***	***0.006***	***0.017***	***0.004***	***0.027***
2015	G x T	8.06	3	6.43	4.82	0.60
	G x TF	8.57	3	6.79	5.11	0.60
	L x T	7.51	3	5.86	4.43	0.59
	L x TF	7.44	3	5.99	4.86	0.66
	Mean	**7.90**	**3**	**6.26**	**4.81**	**0.61**
	Cv (%)	**6.7**	**0.00**	**6.79**	**5.85**	**5.23**
	P Value	***0.548***	***0.712***	***0.761***	***0.834***	***0.238***
2016	G x T	14.43	3	9.31	7.38	0.47
	G x TF	15.49	3	9.60	7.38	0.47
	L x T	13.10	3	8.32	6.07	0.47
	L x TF	13.10	3	8.64	5.67	0.44
	Mean	**14.03**	**3**	**8.97**	**6.63**	**0.46**
	Cv (%)	**8.25**	**0.00**	**6.58**	**13.39**	**3.24**
	P Value	***0.447***	***0.131***	***0.978***	***0.624***	***0.461***

Values in a column differ significantly at p = 0.05 if they are followed by different letters

G = Granular N, L = Liquid N, T = Tillering, TF = Tillering and Flowering

Responses of wheat plants to N rates were generally positive for most parameters in this study. Table 6.8 shows that increasing the amount of N applied generally improved grain yield. In 2014, the grain yield varied from a mean maximum of 11 g pot^{-1} to a minimum of 9 g pot^{-1}. Although there are instances of no effect of increasing N rates on grain yield, the majority of reports suggest that plants generally respond positively to increasing N rates. Nakano et al. (2008) reported a significant improvement of grain yield when N was increased from 0 to 8 g m^{-2} in wheat studies in Japan. Increasing N rate from 0 to 240 kg ha^{-1} at tillering significantly increased grain yield in wheat studies on N rate and time of application in Iran (Abedi et al. 2011).

Table 6.9 shows the responses of grain yield to the method x timing effect. The application of N with liquid N sources at tillering did not differ significantly with the application of granular N as either a single dose at tillering or a split between tillering and flowering. The results however, show that the timing was critical in responses shown by grain yield of liquid N applications. Splitting liquid N application significantly reduced the production of grain compared to single application at active tillering. Emam and Borjian (2000) found that pre-anthesis foliar feeding of wheat resulted into higher grain yields as compared with later application in studies conducted in Shiraz, Iran. Dampney and Salmon (1990) found that timing of foliar N had an impact on grain yield. The authors recorded higher grain improvements when foliar N was applied between swollen (GS 43) and ear ¾ emerged (1.02 and 1.08 t ha^{-1} respectively) and the effect was reduced when foliar N was applied between the end of anthesis and early dough development (0.36 and 0.48 t ha^{-1} respectively). This could be an indication that, for liquid N topdressings to improve grain yield, applications should be conducted before anthesis. These studies are in agreement with the findings of Amanullah et al. (2015) who reported that grain yield increased significantly when N was applied as a single dose 90 days after emergence compared to splitting the same amount of N in different growth stages. Turley et al. (2001) stated that yield responses of foliar N are not likely at later N timing applications (after anthesis), as the capacity for carbohydrate accumulation is diminishing. Morgan (1988) mentioned that early season N application improves the accumulation of dry matter due to enhanced tiller number and larger photosynthetic surface.

6.3.5 Harvest index (HI)

The HI was significantly affected by N treatment in 2014 and 2016. The HI values ranged between 0.24 (Control) and 0.37 (UAN 30 kg N ha^{-1} or split applied granular urea at 30 kg N ha^{-1}) in 2014 (Table 6.6). The trend shows that the variation was not statistically different between the majority of the treatments. Generally, there was a positive relationship between the HI and GY. These results confirm the findings reported by several other authors (Thomson et al. 1997; Rao and Bhagsari 1998; Rao et al. 2002) who reported positive associations of HI and GY. The negligible effect of N treatment of HI in 2015 was probably due to the altered growth and development caused by the fungal disease infection earlier in the season. As can be observed in Table 6.6, the HI values are high, exceeding the 0.6 value considered the limit by Austin et al. (1980). Since N remobilization from the vegetative components account for a greater share of final N in grain (Simpson et al. 1983), it could be argued that fungal infections in 2015 triggered imbalances in N partitioning, which resulted to these high harvest indices. Peltonen-Sainio (1991) indicated that oat cultivars with extremely high HI of about 0.6 were not among the highest yielding ones in genetic improvement studies.

The effect of N rate showed that increasing N rate produced plants with higher HI values compared to lower N rates in 2015 only. Similar findings were reported by Tollenaar et al. (1997), who found that the HI values of maize were low (0.41) where no N was added and increased to high HI values (0.45) at high N rates across two hybrids. Fageria (2007) mentioned that HI values of 10 upland rice genotypes were improved by N fertilisation.

Figure 6.1 shows the interactive effect of timing and N rate on HI was significant in 2016. Although not significant to both N application timings at 60 kg N ha^{-1}, a single application of N at 30 kg ha^{-1} generated significantly higher HI compared to split applications at 30 kg N ha^{-1}. These responses could be ascribed to higher N use efficiencies in single versus split application of N at lower N rates, which probably improved grain yields. Costa et al. (2017) reported high N efficiencies in wheat following single N application compared to split application in studies conducted in Brazil.

The interactive effect of method x timing interaction was not significant as indicated by the Fischer LSD separation of means although the ANOVA p-value was significant (Table 6.9). Throughout this study, the application of liquid at tillering demonstrated better responses compared to split applications of liquid N. A similar trend was observed for HI, indicating the inter-relationships existing between yield components and HI.

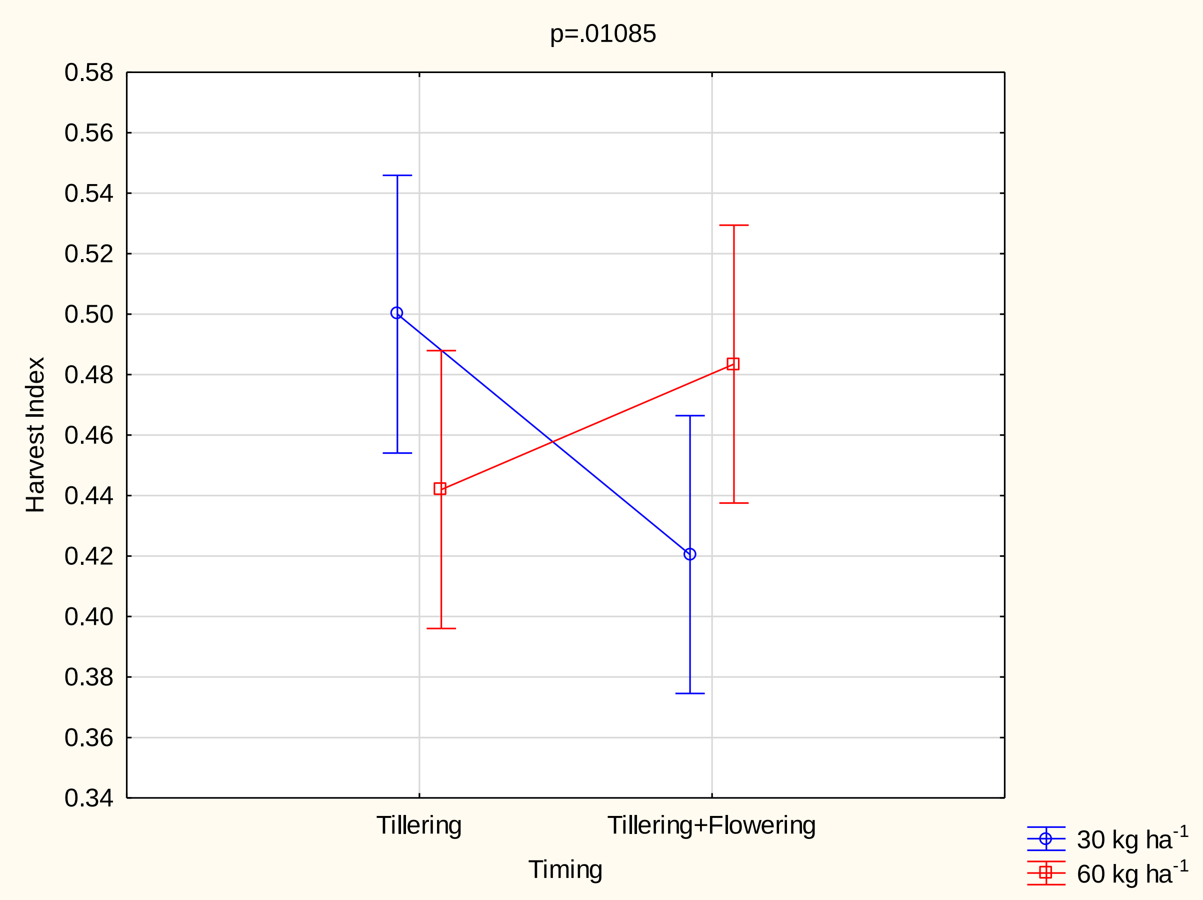

Figure 6.1: Effect of the interaction between N application rate and time of application on harvest (Different letters above bars indicate significant differences at p = 0.05), T = N application at tillering, TF = N application at tillering and flowering

Grain protein content (GPC) and falling number (FN)

The summary of effect of N treatments on grain protein content (GPC) and falling number (FN) is shown in Table 6.11. The treatments did not have a significant effect on GPC and FN. The GPC varied between 9 and 11%. The trends suggest that split applications of N improved GPC compared to single applications although the effect was statistically not significant. To boost GPC, Gooding and Davies (1992) reported that the optimum timing for foliar N is during anthesis or grain milk development (Smith et al. 1987; Clare et al. 1993; Dampney et al. 1995). Pushman and Bingham (1996) reported increasing protein concentration from application of late-season N either as liquid foliar sprays or dry granular topdress fertilisers even though early season N applications were more sufficient for potential grain yield.

Similarly, the FN was not significantly influenced by the nitrogen treatment under these glasshouse conditions (Table 6.11). The FN values ranged between 299 and 360 s from a split applied liquid urea at 15 and 30 kg N ha^{-1} respectively. Compared to GPC values, no clear trend was observed for FN in this study. The FN is an indication of α-amylase activity and measures the breakdown of starch in the kernel through the enzymatic activities (Liniņa and Ruža 2012). Alpha-amylase activity is influenced by weather conditions especially precipitation (Skudra and Liniņa 2011). Knapowski and Ralcewiski (2004) reported increases in the FN with nitrogen applications compared to the control. Although there are reports of effect of N on FN, Smith and Gooding (1996) stated that, the influence of N fertiliser is lower than the effect of cultivar and climatic conditions owing to lack of responses observed in this study. Farrell and Kettlewell (2008) concluded that FN depends significantly on the interaction between cultivar and the conditions at the corresponding location.

Table 6.10: Summary of the effect of N treatments on grain protein content (GPC) and falling number (FN) on wheat grown under glasshouse conditions

Treatment	**GPC (%)**	**FN (s)**
Control	9	325
Granular Urea 30 T	10	341
Granular Urea 15 TF	11	335
Granular Urea 60 T	11	354
Granular Urea 30 TF	11	344
Liquid Urea 30 T	10	322
Liquid Urea 15 TF	11	299
Liquid Urea 60 T	10	350
Liquid Urea 30 TF	11	360
Liquid UAN 30 T	11	317
Liquid UAN 15 TF	11	338
Liquid UAN 60 T	10	300
Liquid UAN 30 TF	11	344
Mean	**10**	**333**
Cv (%)	**5.69**	**5.8**
P Value	***0.990***	***1.000***

UAN = Urea ammonium nitrate, T = applied at tillering, TF = applied at tillering and flowering

6.4 Multiple linear regression analysis

Table 6.11 shows the results of the multiple linear regression analysis. According to the model, plant biomass and number of ears were excluded due to severe multiple collinearity. The model shows that grain yield was significantly affected by mass of ears and to a lesser extent harvest index (Adjusted $R^2 = 0.92$), which explains 92% of the variation in the data. According to the model, if the harvest index is held constant, one unit increase in mass of ears will result in 104% (b* = 1.04) unit increase in grain yield. With mass of ears held constant, grain yield will improve by 18% (b* = 0.18) if harvest index increases by one unit. This analysis expresses the strong influence that mass of ears played to the improvement of grain yield in this study.

Table 6.11: Multiple linear regressions analysis of mass of ears, harvest index, plant biomass and number of heads on grain yield in glasshouses studies

	Regression Summary for Dependent Variable: Grain Yield R= .96106098 R²= .92363821 Adjusted R²= .92284278 CV-R^2=0.92						
N=195	b*	Std.Err.	b	Std.Err.	t(192)	p-value	# times in best 20 models
Intercept			-1.96515	0.324991	-6.04679	0.000000	
Mass of ears	1.041913	0.023385	0.78881	0.017704	44.55498	0.000000	4
Harvest index	0.177899	0.023385	3.28956	0.432414	7.60743	0.000000	4
Plant biomass	Excluded						4
No of ears	Excluded						4

6.4 Conclusions

This study showed that the different growth parameters were significantly affected by the N treatment in 2014 and 2016. Plant growth and development responded positively to increasing N rates. The application of larger amounts of N significantly improved PBPP, NEPP, MEPP, GYPP and HI. The effect of the method of application tended to favour the granular N applications where the effect was significant. The method of N application x timing interaction showed that liquid N applications at tillering were superior compared to split applications. The results also showed that an advantage was gained by split applications of granular N applications compared to single applications. The multiple linear regression analysis showed that grain yield was significantly affected by mass of ears and to a lesser extent, harvest index. Grain protein content and the falling number were not affected by the N fertiliser treatment. However, the trends favoured split applied N over single topdressing for grain protein content but no clear trend was observed for the falling number.

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Chapter 7

Effect of granular and liquid applied nitrogen topdressings on nitrogen use efficiency of spring wheat (*Triticum aestivum* L.) under field and controlled glasshouse conditions

Abstract

Studies were conducted under field and glasshouse conditions to study the effect of granular and liquid applied nitrogen fertiliser topdressing on NUE of spring wheat. The field experiment was conducted at Roodebloem and Langgewens Experimental Farms of the Western Cape province of South Africa for three seasons (2013-2015) under dryland conditions, to evaluate the effect of nitrogen (N) fertiliser topdressing using granular and liquid applied N at two N rates (30 and 60 kg ha^{-1}) . The design was a complete randomised block design with 12 N treatments replicated three times. The N sources were limestone ammonium nitrate (LAN), urea (solution), urea (granular), and liquid urea ammonium nitrate (UAN) applied at early tillering at a rate of 30 and 60 kg N ha^{-1}. At sowing, N was applied as LAN at 30 kg ha^{-1} in all the plots. A similar pot experiment was conducted under glasshouse conditions with the same objectives and the treatments were applied at rates equivalent to 30 and 60 kg N ha^{-1} at tillering. A second glasshouse experiment was conducted to further evaluate the effect of single N applications versus split applications on NUE. The results showed that partial factor productivity (PFP_N) significantly (*p≤0.05*) decreased with increasing N rates in all the experiments. Plant N uptake (straw + grain) increased with increasing N rates where the effect was significant. At Roodebloem, the interaction between the method of N application and N rate showed that PFP_N and nitrogen utilization efficiency (NUtE) were significantly ($p \leq 0.05$) improved by liquid N applications at 30 kg N ha^{-1} compared to other treatments although the effect did not differ with granular applications. The low seasonal rainfall at Langgewens in

2015 reduced the effect of the method x N rate interaction although significant responses were observed for different NUE parameters at treatment level. The multiple linear regression analyses showed that grain N uptake and PFP_N significantly influenced grain yield at both Roodebloem and Langgewens. In the first glasshouse experiment, liquid N applications significantly improved the studied NUE parameters compared to granular applications. The multiple linear regressions confirmed that grain N uptake, straw N uptake and PFP_N significantly affected grain yield compared to other parameters. The second glasshouse experiment revealed that split applications of granular N topdressing and a single liquid N application were superior compared to either single granular or split liquid application in improving wheat NUE. Similarly, the multiple linear regression analysis showed that grain N uptake, straw N uptake and PFP_N significantly affected grain yield.

Keywords: Granular applied N, liquid applied N, nitrogen use efficiency (NUE), partial factor productivity (PFP_N), urea ammonium nitrate (UAN)

7.1 Introduction

Amongst others, nitrogen (N) is the most limiting nutrient element for the production of wheat (Pan et al. 2006). According to Aspuland et al. (2014), nitrogen use efficiency (NUE) plays a significant role in the sustainability of grain production due to the effect of N on wheat production and the possible environmental problems associated with its use. Nitrogen use efficiency is defined as the ratio between grain yield and the amount of nutrient provided by fertiliser (Moll et al. 1982; Cormier et al. 2013; Dai et al. 2013). Mineral N fertiliser accounts for a significant cost in wheat production and is associated with negative impacts on the environment through leaching and N_2O emissions (Arregui and Quemeda 2008; Cui et al. 2014).

Applied N not absorbed by the crop or immobilized in soil organic pools, which include both microbial biomass and soil organic matter is exposed to losses from volatilization, denitrification, and leaching (Cassman et al. 2002), and this may result in harmful pollutants of the environment (Shrawat et al. 2008). Improving N uptake efficiency from different applied N inputs can improve the overall NUE of a cropping system by reducing the amount of N losses from soil organic and inorganic pools or both (Cassman et al. 2002). According to these authors, wheat responds positively to N fertilization, with significant amounts of supplemental N required to improve grain yields. However, only a small amount (30-35%) of the applied N is absorbed and utilized by plants in the year of application (Raun and Johnson 1999). Gupta and Khosla (2012) later reported that NUE for most cereal production systems is between 40 and 50%. This is a notable improvement of 33% from the late 1990s estimates by Raun and Johnson (1999) and is largely attributed to continuous advances in fertiliser management strategies and novel fertiliser technologies (Walsh and Christians 2016).

The broadest measure of NUE is the ratio of yield to amount of applied N, also referred to as partial factor productivity (PFP_N) (Dobermann 2005). According to Dobermann (2005), PFP_N is an aggregate efficiency index that considers the contributions to crop yields from uptake of soil residual N, N fertilizer uptake efficiency and the efficiency with which N is acquired and translocated into grain yield by the plant. Nitrogen utilization efficiency (NUtE) and nitrogen harvest index (NHI) are amongst some of the commonly used NUE indices and the choice of which NUE index to use is mostly influenced by the intended purpose and the available resources. Nitrogen harvest index (NHI) is the ratio between N uptrake in grain and N uptake in grain plus straw or shoot (Fageria 2014). The NHI is an important index that determines the retranslocation efficiency of absorbed N from vegetative plant parts to the grain. The NHI measures N partitioning in crop plants which gives an indication of how efficiently the plant utilize acquired N for grain production (Fageria and Baligar 2003).

The objective of modern agriculture is to reduce the negative impact of cropping systems on the environment, improve the quality of crop products, produce crops at low cost and improve N use efficiency (Gastal and Lemaire 2002). Management practices that would assist farmers to increase productivity at a low cost should be studied to ensure sustainable agricultural production. Wheat remains one of the most important cereal crops in South Africa. At the same time, N is the element that contributes significantly to wheat grain yields and grain protein content. Currently, there is limited information locally on NUE in wheat, particularly in studies involving the use of liquid applied nitrogen fertilisers under dryland conditions. The aim of this study was to evaluate the effect of granular and liquid applied N on NUE of spring wheat under field and controlled glasshouse conditions.

7.2 Materials and methods

The details of the experimental design, soil and water analyses, planting, fertilization and harvesting procedures are shown in Chapter 4, 5 and 6 of this thesis, for Section A, B and C respectively. The treatments applied are shown in Table 7.1, 7.2 and 7.3 for Section A, B and C respectively. For the field experiment, only 2014 and 2015 data was used for Roodebloem and only 2015 for Langgewens. The plant biomass was not sampled in 2013 at both localities hence the exclusion. On the one hand, the protein content data for 2014 at Langgewens was misplaced by the analytical laboratory and could not be registered. For Section C, the 2015 experimental data was excluded because the protein content was not analysed as the harvested grain failed to meet the minimum mass of grain (g) required for protein content analysis and therefore N grain uptake could not be calculated.

At physiological maturity, the above-ground biomass samples (1 m^2 area for field experiment and per pot in glasshouse experiments) were collected, dried (60˚C for 48 hours) and weighed. Grain was separated from the residue, air cleaned and weighed to determine the grain yield. The straw (residue) total N concentration was determined with a Leco-combustion analyser (Leco FP-2000, Leco Corporation, St Joseph, MI, USA) at BemLab (Pty) Ltd, Strand, South Africa and at Döhne Agricultural Development Institute, Stutterheim, South Africa.

Grain protein content was determined using the Near-Infrared Reflectance method for protein in wheat flour using the AACC Method 39-11 (AACC 2000) at Welgevallen Experimental Farm of the University of Stellenbosch, Stellenbosch, South Africa. To calculate grain N concentration, grain protein content (%) from each treatment was divided by the Jones factor (5.83) (Jones 1941). Wheat grain N uptake and straw N uptake were calculated by multiplying the grain N (%) and straw N (%) by the grain and straw yield respectively. The total N uptake was the summation of grain N uptake and straw N uptake.

Table 7.1: Nitrogen fertiliser treatments for field experiments

Treatment	Method	Sowing (LAN) (kg ha^{-1})	Tillering (kg ha^{-1})	Total N (kg ha^{-1})
0 N	0	30	0	30
LAN 30+	0	30	30 + Activate N	60
LAN 60+	0	30	60 + Activate N	90
LAN 30	Granular	30	30	60
LAN 60	Granular	30	60	90
Urea 30	Granular	30	30	60
Urea 60	Granular	30	30	90
Urea 30	Liquid	30	30	60
Urea 60	Liquid	30	60	90
UAN 30	Liquid	30	30	60
UAN 60	Liquid	30	60	90

LAN = Limestone Ammonium Nitrate (28%), UAN = Urea Ammonium Nitrate (32%), LAN 30/60+ = LAN was followed by a foliar application of Activate N

The partial factor productivity of N (PFP_N)N utilisation efficiency (NUtE.), N harvest index (NHI) and were calculated following Moll et al. (1982), Craswell and Goldwin (1984), Doyle and Holford (1993), Baligar et al. (2001) and Ladha et al. (2005).

PFP_N = GY/N applied = kg kg^{-1}

NUtE. = GY/NT uptake = kg kg^{-1}

NHI = NG/NT

Where GY is grain yield, NG is total N in grain, NT is the total N uptake.

Table 7.2: Nitrogen topressing treatments in the Glasshouse Experiment 1 in 2013, 2014 and 2016

Treatment	Method	Sowing (LAN) (g pot^{-1})	Tillering
Control	0	0	0
LAN 30+*	0	0.32	0.32 g pot^{-1}
LAN 30	Granular	0.32	0.32 g pot^{-1}
LAN 60	Granular	0.32	0.64 g pot^{-1}
Urea 30	Granular	0.32	0.19 g pot^{-1}
Urea 60	Granular	0.32	0.39 g pot^{-1}
Urea 30	Liquid	0.32	81.5 g 500 ml^{-1}
Urea 60	Liquid	0.32	163 g 500 ml^{-1}
UAN 30	Liquid	0.32	88.75 ml 500 ml^{-1}
UAN 60	Liquid	0.32	177 ml 500 ml^{-1}

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate, + = LAN followed by foliar application of Activate N, *LAN 30+ = was substituted with UAN 90 in 2016

A one-way analysis of variance (ANOVA) was performed using the GLM (General Linear Model) Procedures of the Statistica Software 13.2 test for differences between treatments for all parameters (StatSoft Inc. Tulsa, OK, USA). Means were separated using the Fisher's protected least significant difference (LSD) test at *p=0.05*. In cases where residuals were not normally distributed, the Kruskal-Wallis test was used as a non-parametric test to confirm the results of the ANOVA. In cases where Levene's test for homogeneity of variances indicated heterogeneous variances, the LSD test was replaced with the Games-Howell multiple comparison procedure. For the field experiment (SECTION A), to analyse the effect of N rate, method of N application, and their interactions; data sets from granular urea and granular LAN (Combined as granular N), and liquid urea and UAN (Combined as liquid N) at both 30 and 60 kg ha^{-1} were extracted and analysed separately as a 2x2 factorial experiment repeated three times.

Table 7.3: Nitrogen topdressing treatments in the Glasshouse Experiment 2 in 2014 and 2016

Treatment	Method	Sowing (LAN) g pot^{-1}	Tillering	Flowering
Control	0	0	0	0
Urea 30 Single	Granular	0.21	0.13 gpot^{-1}	0
Urea 30 Split	Granular	0.21	0.07 g pot^{-1}	0.07 g pot^{-1}
Urea 60 Single	Granular	0.21	0.26 g pot^{-1}	0
Urea 60 Split	Granular	0.21	0.13 g pot^{-1}	0.13 g pot^{-1}
Urea 30 Single	Liquid	0.21	81.5 g 500 ml^{-1}	0
Urea 30 Split	Liquid	0.21	40.75 g 500 ml^{-1}	40.75 g 500 ml^{-1}
Urea 60 Single	Liquid	0.21	163 g 500 ml^{-1}	0
Urea 60 Split	Liquid	0.21	81.5 g 500 ml^{-1}	81.5 g 500 ml^{-1}
UAN 30 Single	Liquid	0.21	88.75 ml 500 ml^{-1}	0
UAN 30 Split	Liquid	0.21	44.38 ml 500 ml^{-1}	44.38 ml 500 ml^{-1}
UAN 60 Single	Liquid	0.21	177.5 ml 500 ml^{-1}	0
UAN 60 Split	Liquid	0.21	88.75 ml 500 ml^{-1}	88.75 ml 500 ml^{-1}

In the Glasshouse Experiment 1 (Section B), the effect of N rate, N application method and their interactions were analysed by extracting the data sets from granular urea and granular LAN (combined as granular N) and liquid urea and UAN (combined as liquid N) at 30 and 60 kg ha^{-1} and analysing it as a 2x2 factorial experiment replicated six times.

The effect of N timing, N rate, N method and the interactions in the second glasshouse experiment (Section C) was analysed by extracting data sets from granular N treatments and liquid N treatments applied at 30 and 60 kg ha^{-1} once at tillering or twice between tillering and early anthesis. The data sets were analysed as a 2x2x2 factorial experiment replicated five times. The N rate analyses was performed at 30 and 60 kg ha^{-1} topdressing level. The N rate analyses considered the total amount of N topdressing irrespective of the time of application. This means that the two applications of N at 15 kg ha^{-1} were analysed as 30 kg N ha^{-1} topdressing rate together with a single topdressing of N at 30 kg ha^{-1}. The single application of N at 60 kg ha^{-1} and the split applications of N at 30 kg ha^{-1} between tillering and early anthesis accounted for 60 kg ha^{-1} N rate. The variance estimation, precision and comparison (VEPAC) package of Statistica 13.2 was used for statistical analyses.

Multiple regression analysis was performed for all the tested variables and grain yield was the dependant variable to assess which variables contributed significantly to the grain yield and to further evaluate the relationship between the different variables. Where there was severe multi-collinearity among the input variables, the best five input variables were selected using the best subsets procedure and variables were then reduced to the best three or two input variables.

7.3 Results and discussion

7.3.1 SECTION A – Field conducted studies

The results showed that all the studied parameters were significantly influenced ($p \leq 0.05$) by N treatment at Roodebloem in 2014 (Table 7.4). In 2015, the PFP_N and N utilization efficiency were significantly affected by the N treatments.

The effect of N rate showed a significant effect on PFP_N in both years at Roodebloem, however, no significant response was observed for other parameters (Table 7.5). Grain N uptake, nitrogen harvest index (NHI) and N utilization efficiency were significantly affected by the interaction between N rate and method of application in 2014.

At Langgewens, all the measured NUE parameters were significantly affected by N treatments with the exception of grain N uptake (Table 7.4). As observed at Roodebloem, the PFP_N responded significantly to the application of N rate and the NHI was affected by the interaction between the N rate and the method of N application (Table 7.5).

Table 7.4: Analysis of variance (AVOVA) results for partial factor productivity, grain uptake, straw N uptake and total N uptake, nitrogen harvest index (NHI), nitrogen utilization efficiency (NUtE) and apparent nitrogen recovery at Roodebloem and Langgewens

		Source					
		N treatments					
Roodebloem	**Year**	**PFP_N**	**Grain N uptake**	**Straw N uptake**	**N Total uptake**	**NHI**	**NUtE**
	2014	*	*	*	*	*	*
	2015	*	ns	ns	ns	ns	*
Langgewens	**Year**	**PFP_N**	**Grain N**	**Straw N**	**N Total**	**NHI**	**NUtE**
	2015	*	ns	*	*	*	*

* Significant at the 0.05 probability level

ns = not significant at the 0.05 probability level

Table 7.5: Analysis of variance (ANOVA) results for the effect of N rate, N method of application, N rate x N method on PFP_N, grain, straw and total N uptake, NHI and NUtE at Roodebloem and Langgewens

Roodebloem	**Source**	**PFP_N**	**Grain N uptake**	**Straw N uptake**	**N Total uptake**	**NHI**	**NUtE**
2014	N rate	*	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns	ns
	N rate x Method	ns	*	ns	ns	*	*
2015	N rate	*	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns	ns
	N rate x Method	ns	ns	ns	ns	ns	ns
Langgewens	**Source**	**PFP_N**	**Grain N uptake**	**Straw N uptake**	**N Total uptake**	**NHI**	**NUtE**
2015	N rate	*	ns	ns	ns	ns	ns
	Method	ns	ns	ns	ns	ns	ns
	N rate x Method	ns	ns	ns	ns	*	ns

* Significant at the 0.05 probability level

7.3.1.1 Grain N uptake, straw N uptake and total N uptake

Grain N uptake was significantly (*p≤0.05*) affected by the N treatment in 2014 but not in 2015 at Roodebloem. Limestone ammonium nitrate (LAN) applied with Activate N (LAN 60+) produced the highest mean grain N compared to other treatments. Table 7.6 shows that grain N uptake ranged between 70.07 kg ha^{-1} (LAN 60+) and 52.4 kg ha^{-1} (Urea L 60). An improved N uptake in LAN 60+ could be due to the ability of the applied inoculant to fix N. Following LAN application, a product containing a mixture of *Bacillus* spp. and *Herbaspirillum* spp. plant growth promoting inoculant was sprayed on the foliage. According to Baneix et al. (2005), inoculation of wheat with three different species of *Bacillus sp*. consistently increased grain quality and N use efficiency. Garcia de Salamone et al. (2012) and Pereg et al. (2016) attributed the beneficial effects of the inoculation to increased root development, which improved rates of water and mineral uptake. Herrera et al. (2016) stated that, the indirect effect of these plant growth-promoting inoculants is associated with the ability to protect plants against phytopathogens. The grain N uptake ranged between 69.82 and 81.6 kg ha^{-1} in 2015.

In 2014, the interaction between N rate and method of application showed that granular N applications at 30 kg ha^{-1} significantly (*$p \leq 0.05$*) improved grain N uptake compared with granular applied N at 30 kg ha^{-1} and liquid N applications at 60 kg ha^{-1} but not significantly so to granular applications at 60 kg ha^{-1} (Table 7.8). Grain N uptake due to this interaction ranged between 65.22 (liquid N at 30 kg ha^{-1}) and 55.85 kg ha^{-1} (liquid N at 60 kg ha^{-1}). No significant effect of this interaction was observed in 2015. Higher grain N uptake from liquid N applications could be attributed to the increased permeability of the cuticle, especially that of liquid urea, which improves N diffusion into the leaf (Franke 1967).

Table 7.6 shows that straw N uptake was significantly affected by N treatment in 2014. Although no clear trend was observed, the straw N uptake to a certain extent was enhanced by higher N application rates. The highest straw N uptake was obtained in plants top-dressed with urea at 60 kg N ha^{-1} (68.93 kg N ha^{-1}), while liquid urea (30 kg ha) showed the lowest straw N uptake (39.91 kg N ha^{-1}). The effect of N rate showed that applications of N at 60 kg ha^{-1} significantly improved straw N uptake (55.96 kg ha^{-1}) compared to applications of N at 30 kg N ha^{-1} (50.26 kg N ha^{-1}), confirming the observations reported above on the effect of N treatment. Maali and Agenbag (2003) observed an increase in plant N uptake with increasing N topdressing rate from 60 kg N ha^{-1} to 140 kg N ha^{-1} at measurements taken at anthesis in Langgewens. The authors, however, also noted a relatively higher plant N uptake (196 and 169 kg N ha^{-1}) for the 60 kg N ha^{-1} treatment and ascribed these to the contribution made by N-mineralisation to the supply of N for these crops. Since, the total N uptake is a summation of grain N and straw N uptake, the trends generally followed similar patterns as observed with grain and straw N.

Grain N uptake was not significantly affected by N treatments at Langgewens in 2015. The general responses of the different parameters are shown in Table 7.7 and as mentioned above, the parameters showing non-significant responses will not be discussed due to the size of this chapter. Although there are significant, differences for other parameters, Langgewens received low total rainfall in 2015 and this probably reduced the effect of applied N on plant growth and development explaining the low responses on N uptake in particular. Fricke et al. (1997) reported that conditions of soil water deficit reduce N uptake by roots and leaves of N deficient plants become fewer with smaller cells and this may be the causal effect of non-responses in this locality.

7.3.1.2 Nitrogen harvest index (NHI)

The statistical data showed that NHI was significantly ($p \leq 0.05$) affected by various N treatments at Roodebloem in 2014 (Table 7.6). The NHI varied between 0.48 (UAN 60) and 0.63 (Control). The trend suggest that increasing N rate had a reducing effect on NHI. As such, the pattern observed for these NHI values was somewhat similar to the responses shown by grain yield in Chapter 4 of this thesis. Plants that did not receive any N during the 2014 season produced the highest mean grain yield compared to other treatments. These responses could be attributed to the relatively low translocation of N from the vegetative organs to the grain, which consequently reduced NHI in the case of fertilised plots. Wang et al. (2003), who found that the ratio of N absorbed from fertiliser to total absorbed N was higher when wheat was grown under low fertility conditions compared to high fertility soil, confirmed similar findings. This was associated with N remaining in the vegetative organs after flowering thereby reducing NUE. The interaction between N rate and method of application showed that liquid N applications at 30 kg ha^{-1} produced the highest mean NHI and was significantly ($p \leq 0.05$) greater than granular applied N at 30 kg ha^{-1} and liquid N at 60 kg ha^{-1}. This interaction was generally consistent for the majority of NUE indices at Roodebloem in 2014.

7.3.1.3 Partial factor productivity (PFP_N)

The results of this study showed that PFP_N ranged between 31.30 kg kg^{-1} and 110.6 kg kg^{-1} at Roodebloem in 2014 from 60 kg N ha^{-1} of urea solution and 0 N topdressing respectively (Table 7.6). A similar trend was observed in 2015, with lowest mean PFP_N (39.33 kg kg^{-1}) found at a higher N application rate (liquid urea at 60 kg N ha^{-1}) and a maximum (110.60 kg kg^{-1}) produced at the lowest N rate (0 N). There was a consistent decrease in PFP_N with increasing N topdressing rate. Since PFP_N is a ratio between harvested grain yield and applied N, it would be expected that this NUE parameter would decrease with increasing N rate. Studies by Ayadi et al. (2016) under rain-fed Mediterranean conditions of Tunisia revealed that PFP_N decreased with increasing N rates in durum wheat genotypes. Although the results were preliminary, Labuschagne (2016) reported similar findings at Riversdale, where PFP_N decreased exponentially with increasing N topdressing rate and the values ranged between 25.5 kg kg^{-1} and 136 kg kg^{-1} from 165 kg N ha^{-1} and 0 N ha^{-1} topdressings respectively.

The effect of N rate revealed that 30 kg ha^{-1} N topdressing produced higher PFP_N compared to 60 kg ha^{-1} N topdressing (Data not shown). In 2014, topdressing wheat with 30 kg N ha^{-1} (52.7 kg kg^{-1}) improved PFP_N by 35% compared to topdressing wheat with 60 kg N ha^{-1} (34 kg kg^{-1}). Similarly, 58.34 kg kg^{-1} was produced following the applications of N topdressings at 30 kg ha^{-1} compared to 39.3 kg kg^{-1} obtained with topdressings at 60 kg ha^{-1} in 2015. Zhu et al. (2011) found that PFP_N declined significantly with the increase in the amount of N applied in spring wheat studies conducted in China. Panayotova and Kostadinova (2015) suggested that typical PFP_N values were ranging between 40 and 80 kg kg^{-1}.

The interactive effect between N rate and the method of N application is shown in Table 7.8. Liquid N applications at 30 kg N ha^{-1} at tillering resulted into significantly ($p \le 0.05$) higher mean PFP_N (55.32 kg kg^{-1}) compared to other treatments. However, in 2015, no significant interactions were observed. Dobermann (2005) reported PFP_N values ranging from 245 kg kg^{-1} in the period between 1961 and 1965, 52 kg kg^{-1} between 1981 and 1985 to a recently reported 44 kg kg^{-1} for most developed countries. The author attributed the improvement in PFP_N to a combination of using fertile soils, conducive climatic conditions and improved management practices.

Due to technical challenges experienced during this study, only 2015 results are reported for Langgewens. Table 7.7 shows that PFP_N was significantly affected by the N treatment in 2015. The effect was generally similar to that observed at Roodebloem, with PFP_N showing an exponential decrease with increasing N rate although the responses tended to be lower than at Roodebloem. This could be ascribed to drought conditions experienced in this sub-region in 2015, which reduced grain yields. Partial factor productivity is a function of grain yields and N applied, and therefore, any conditions that limit crop growth and development will result in the reduction of PFP_N. Hoseinlou et al. (2013) found that PFP_N was reduced significantly, as drought stress increased in NUE studies under water deficient conditions in spring barley. Under drought conditions, higher amounts of N reduce C/N ratio, and nitrate absorption is induced, resulting into a saturation of N metabolism, which impose adverse effect on NUE (Jiang and Hull 1998). The PFP_N ranged between 10.6 kg kg^{-1} (LAN 60+) and 49.87 kg kg^{-1} (0N).The effect of N rate revealed that PFP_N increased with decreasing N rate. A significantly higher mean PFP_N of 20.97 kg kg^{-1} was produced following the application of N topdressings at 30 kg ha^{-1} compared to 12.59 kg kg^{-1} found with 60 kg N ha^{-1}(Results not shown). No N rate x method of N application interactions were observed.

Table 7.6: Effect of N treat on PFP_N, grain N uptake , straw N uptake and total N uptake, NHI and NUtE at Roodebloem

	PFP_N (kg kg^{-1})		Grain N uptake (kg ha^{-1})		Straw N utake (kg ha^{-1})		N Total uptake (kg ha^{-1})		NHI		NUtE (kg ha^{-1})	
Treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	110.60a	110.60a	60.54bcde	70.44	43.93efg	17.37	104.47ef	87.81	0.58ab	0.48	31.75b	38.81
LAN 30+	52.23b	55.27b	60.99bcd	69.82	68.48a	28.53	129.46ab	98.35	0.47cde	0.36	24.22ef	33.82
LAN 60+	37.60c	39.70c	70.07a	81.51	54.20cd	35.09	124.27bc	116.60	0.56ab	0.47	27.18cde	30.92
Granular LAN 30	53.53b	57.73b	63.67abc	78.40	57.67bc	28.34	121.34e	106.74	0.52ab	0.37	26.48de	32.63
Granular LAN 60	36.57c	38.60c	60.57bcde	81.60	42.03efg	18.91	102.60ef	100.51	0.59ab	0.40	32.03b	34.54
Granular Urea 30	48.15b	59.45b	54.22de	78.26	56.73bcd	27.45	110.95de	105.71	0.49e	0.37	26.00def	33.76
Granular Urea 60	34.23c	39.70c	64.93abc	80.82	68.93a	23.84	133.86a	104.66	0.49bcd	0.46	23.04f	35.20
Liquid Urea 30	54.17b	57.77b	62.69abcd	75.40	39.91fg	19.98	102.60ef	95.38	0.61ab	0.47	31.68b	37.28
Liquid Urea 60	31.30c	39.33c	52.40e	77.61	49.43dc	13.20	101.84f	90.81	0.52e	0.40	27.70cd	39.06
Liquid UAN 30	56.47b	58.13b	67.75ab	77.10	45.19ef	30.45	112.95d	107.54	0.60ab	0.37	29.97bc	32.88
Liquid UAN 60	34.13c	39.60c	59.30cde	77.52	63.47ab	5.42	122.77bc	82.94	0.48e	0.64	25.07def	43.18
Mean	**49.91**	**54.17**	**61.56**	**77.13**	**53.63**	**22.60**	**115.19**	**99.73**	**0.54de**	**0.44**	**27.74**	**35.64**
Cv (%)	**44.51**	**38.50**	**8.12**	**5.54**	**21.27**	**36.27**	**10.54**	**9.48**	**10.37**	**19.47**	**27.63**	**9.80**
P Value	***0.010***	***0.010***	***0.007***	***0.090***	***0.010***	***0.057***	***0.029***	***0.070***	***0.010***	***0.750***	***0.010***	***0.140***

Values within a column differ significantly at *p=0.05* if they are followed by different letters

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate + = LAN followed by a foliar application of Activate N

Table 7.7: Effect of N treatment on PFP_N, grain N uptake, straw N uptake, total N uptake, NHI andNUtE at Langgewens in 2015

Treatment	PFP_N	Grain N uptake	Straw N uptake	N Total uptake	NHI	NUtE
	(kg kg^{-1})					(kg kg^{-1})
Control	49.87a	40.66	12.77cd	53.44ab	0.75abc	27.63bc
LAN 30+	17.73bc	27.43	3.40e	30.83d	0.89a	34.38a
LAN 60+	10.60c	26.01	3.88e	29.89b	0.85ab	31.24ab
Granular LAN 30	21.27bc	35.39	9.07de	44.46bcd	0.76ab	27.55bc
Granular LAN 60	14.50bc	39.26	13.99cd	53.25ab	0.73bcd	24.21cd
Granular Urea 30	17.80bc	29.74	24.44ab	54.18ab	0.57de	20.54d
Granular Urea 60	12.57bc	29.19	18.14bc	47.33bc	0.61cde	23.61cd
Liquid Urea 30	22.60b	37.57	29.71a	67.28a	0.56e	20.13d
Liquid Urea 60	11.10bc	27.7	4.71e	32.41cd	0.85ab	30.82ab
Liquid UAN 30	16.53bc	26.78	7.84e	34.63cd	0.77ab	28.48bc
Liquid UAN 60	12.20bc	32.25	8.37de	40.62bcd	0.79ab	27.00bc
Mean	**18.80**	**32.00**	**12.39**	**44.39**	**0.74**	**26.71**
Cv (%)	***58.77***	***17.54***	***71.14***	***27.99***	***14.7***	***16.39***
P Value	***0.030***	***0.220***	***0.010***	***0.030***	***0.022***	***0.024***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

LAN = Limestone ammonium nitrate, UAN = Urea ammonium nitrate, + = LAN followed by a foliar application of Activate N

7.3.1.4 Nitrogen utilization efficiency (NUtE)

The N utilization efficiency (NUtE); expressed as grain yield per unit of above ground biomass N varied between 23.04 kg grain kg^{-1} of applied N and 35.48 kg kg^{-1} (Table 7.6). Increasing N topdressing rate from 0 to 60 kg ha^{-1} reduced NUtE. A similar effect was confirmed by Szmigiel et al. (2016), who reported that increasing N rate from 0 – 150 kg ha^{-1} decreased NUtE by 22.1 kg kg^{-1} in wheat. Similarly, Delogu et al. (1998) and López-Bellido and López-Bellido (2001) demonstrated a linear decrease in NUE with increasing N rates in wheat. These findings suggest that increasing N rate may not always be justified, as plant responses to N vary largely due to prevailing climatic conditions and N-mineralisation (Maali and Agenbag 2003). Huggins and Pan (1993) also reported that NUtEs often decrease with increasing N rates. No effect of N treatment was observed in 2015.

The interaction between N rate x method of application revealed that liquid N applications at 30 kg N ha^{-1} significantly improved NUtE compared to granular N at 30 kg ha^{-1} and liquid N at 60 kg ha^{-1} but was not significantly different to granular N topdressings at 60 kg ha^{-1} as shown in Table 7.8. Higher NUtE from liquid N at 30 kg ha^{-1} could be ascribed to reduced N volatilization losses compared to other interactions. A study by Below et al. (1985) found that foliar N taken up in leaves was mobilised rapidly to grain from pre- and post anthesis applications and were not stored in stem reserves. Hopkinson (1998) also revealed that, labelled N applied to the flag leaf was rapidly transported to the whole plant within 96 hours, confirming the differences through which N is mobilised from different application methods.

At Langgewens, the NUtE ranged between 20.13 kg kg^{-1} and 34.38 kg kg^{-1} in 2015 (Table 7.7). Applications of LAN at 30 kg N ha^{-1} with Activate N (LAN 30+) differed significantly ($p \leq 0.05$) from other treatments but not significantly so to the control (31.76 kg kg^{-1}), LAN 60+ (31.24 kg kg^{-1}) and liquid urea at 60 kg ha^{-1} (30.82 kg kg^{-1}). The harsh climatic conditions that prevailed in this locality in 2015 probably diluted the effect of N treatment, hence no clear trend could be observed in Table 7.7. According to Waraich et al. (2011), water stress causes a depression in nutrient uptake especially N, which may contribute to grain yield. A decline of water within the plant below the threshold level induces stomatal closure, which causes a decrease in transpiration and eventually a reduction in water transport through the plant (North and Nobel 1997). North and Nobel (1997) further indicated that severe drought induces root shrinkage and causes loss of soil-root contact, thereby decreasing the transport of nutrient to the root surface. This could be true for granular soil applied N in 2015, while a lengthened closure of stomata may have reduced the uptake of liquid applied N.

Table 7.8: Effect of the interaction between N method and rate of N application on PFP_N, NUtE, grain N uptake, straw N uptake, total N uptake and NHI at Roodebloem

Effect	**PFP_N**		**NUtE**		**N grain**		**N straw**		**N total**		**NHI**	
	kg.kg^{-1}				**kg ha^{-1}**							
Method x rate	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Granular 30	50.08b	58.73	25.86b	35.05	58.10bc	78.51	57.96a	23.54	116.06	102.05	0.50b	0.43
Granular 60	35.40c	39.15	27.54ab	34.87	62.75ab	81.21	55.48a	21.37	118.23	102.59	0.54b	0.43
Liquid 30	55.32a	57.95	30.82a	35.08	65.22a	76.25	42.55b	25.21	107.77	101.46	0.61a	0.42
Liquid 60	32.72c	39.47	26.39b	41.12	55.85c	77.56	56.45a	9.31	112.30	86.88	0.50b	0.52
Mean	**43.38**	**48.83**	**27.65**	**36.53**	**60.48**	**78.38**	**53.11**	**19.86**	**113.59**	**98.24**	**0.54**	**0.45**
Cv (%)	**25.42**	**22.51**	**8.05**	**8.38**	**7.06**	**2.68**	**13.39**	**36.29**	**4.04**	**7.73**	**9.65**	**10.42**
P Value	***0.016***	***0.540***	***0.022***	***0.127***	***0.005***	***0.634***	***0.042***	***0.196***	***0.809***	***0.204***	***0.000***	***0.451***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

7.3.1.6 Multiple regression analysis for Roodebloem

Table 7.9 shows the multiple regression analysis of different N use efficiency parameters with grain identified as the dependant variable. Due to severe multi-collinearity that existed among the different predictor variables, straw N uptake, total N uptake, NHI, NUtE, and ANR were excluded from the multiple regression analysis as shown in Table 7.9. Grain yield was significantly influenced by PFP_N and grain N uptake, which explained 83% of the variation (Adjusted R^2=0.83). In this model, if PFP_N is controlled, a one unit increase in grain N uptake will result in 89% (b* = 0.89) unit increase in grain yield. If grain N uptake is held constant, a one-unit increase in PFP_N would increase grain yield by 15% (b* = 0.15) units.

Table 7.9: Multiple regression analysis of PFP_N, grain N uptake, straw N uptake, total N uptake, NHI, NUtE and ANR on grain yield at Roodebloem

	Regression Summary for Dependent Variable: Grain yield R= .91272584 R^2= .83306845 Adjusted R^2= .82776904						
N=33	b*	Std.Err.	b	Std.Err.	t(30)	p-value	# times in best 20 models
Intercept			1392.683	110.9476	12.55262	0.000000	
PFP_N	0.154737	0.051500	2.134	0.7104	3.00460	0.003813	6
Grain N Uptake	0.894742	0.051500	26.255	1.5112	17.37366	0.000000	6
Straw N Uptake	Excluded						6
Total N uptake	Excluded						5
NHI	Excluded						6
NUtE	Excluded						6

7.3.2 SECTION B - GLASSHOUSE EXPERIMENT 1

Table 7.10 shows that all the measured NUE parameters were significantly (*p≤0.05*) affected by N treatment under controlled glasshouse conditions throughout the duration of this study (2013, 2014 and 2016).

The responses from the interactive effect of N rate and method of N application generally showed few statistically significant effects. Table 7.11 shows that grain N uptake and total N uptake were significantly influenced by interactions in 2013, while PFP_N and NHI were the only parameters that responded significantly in 2016.

Almost all the tested parameters were significantly influenced by the N rate in 2013, however, NHI showed no significant responses (Table 7.11). In 2014, all the parameters were significantly affected by the N rate with the exception of straw N uptake and NHI. In 2016, only PFP_N, straw N and NHI responded significantly to the effect of N rate.

The effect of the method of N application followed a similar trend as N rate, however, there was less responses in 2016 as shown in Table 7.11.

Table 7.10: Analysis of variance (ANOVA) for PFP_N, , grain N uptake, straw N uptake, total N uptake and NHI under controlled glasshouse conditions in Glasshouse Experiment 1

	Source		
	N treatment		
Factor	**2013**	**2014**	**2016**
PFP_N	*	*	*
Straw N uptake	*	*	*
Grain N uptake	*	*	*
Total N uptake	*	*	*
NHI	*	*	*

* Significant at the 0.05 probability level

Table 7.11: Analysis of variation (ANOVA) for the effect of N rate, method of application and their interactions for PFP_N, grain N uptake, straw N uptake, total N uptake and NHI under controlled glasshouse conditions in Glasshouse Experiment 1

	Source								
	N rate			Method			N rate x Method		
Factor	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**
PFP_N	*	*	ns	*	*	ns	ns	ns	*
Straw N uptake	*	ns	*	*	ns	ns	ns	ns	ns
Grain N uptake	*	*	ns	*	*	ns	*	ns	ns
Total N uptake	*	*	ns	*	*	ns	*	ns	ns
NHI	ns	*	ns	ns	ns	*	ns	ns	*

* Significant at the 0.05 probability level

ns = not significant at the 0.05 probability level

7.3.2.1 Grain N uptake, straw N uptake and total N uptake

The results of this study showed that grain N, straw N and total plant N uptake generally increased with increasing N rate. In terms of grain N, the control treatment showed the lowest grain N uptake at least in two (2013 and 2014) of the three study years as expected (Table 7.12). Applying UAN at 60 kg ha^{-1} at tillering showed consistency compared to other treatments in improving the uptake of N in grain. These responses were probably due to the ability of liquid applied N to be absorbed much quicker compared to granular applied N. Bowman and Paul (1990) found that a significant portion (30-35%) of N applied through the foliage was absorbed more readily by plants withon12 hours compared to soil applied N.

The effect of the interaction between N rate and method of N application is shown in Table 7.13, while that of N rate is shown in Table 7.14. In 2013, the effect of the interaction between N rate and the method of N application showed that grain N uptake was significantly ($p \leq 0.05$) improved by liquid N application compared to granular N application and the effect was strongly associated with the rate of application (Table 7.15). The trend suggest that liquid N applications resulted in significantly higher grain N uptake when applied at 60 kg N ha^{-1} compared with other treatments. At lower N rates of 30 kg N ha^{-1}, liquid N applications significantly improved N uptake compared to granular N application at similar N rates but the responses were significantly less compared to granular applications at 60 kg ha^{-1}. Throughout the duration of this study, mean grain N uptake increased with increasing N rate. Zhao and Si (2015) reported that applications of 150 kg N ha^{-1} significantly improved grain N uptake compared to the control treatment, while further increases of N topdressing above 150 kg ha^{-1} further improved grain N uptake. Earlier Wittwer et al. (1963) found that the absorption of urea applied as a liquid through foliage was greater compared to N applied as granular fertiliser, which could explain the responses observed in the N rate x method interaction in 2013.

The effect of the method of N application on grain N uptake was significant in 2014. Liquid N application increased N uptake in grain compared to granular soil applied N as shown in Table 7.15. This could be due to higher N recoveries associated with foliar applications compared to soil applications because of reduction of losses. According to Turley et al. (2001), loss processes of foliar applications are less compared to those associated with soil applications. Powlson et al. (1989) and Dampney et al. (1995) found that recoveries of N in grain were increased following foliar applications compared to soil applied N.

The responses of plants' straw N and total N uptake generally followed similar patterns as was observed with the grain N uptake. Straw N uptake increased with increasing N rate (2013) and there was a slight non-significant decrease in 2016 (Table 7.14). The effect of the interaction between the N rate and the method of N application showed that liquid N applications at 60 kg ha^{-1} significantly improved the total N uptake compared to other treatments (Table 7.13). The response pattern was generally similar to the responses shown by grain N uptake as discussed above.

7.3.2.2 Nitrogen harvest index (NHI)

Nitrogen harvest index was significantly affected by N treatment in 2014 (Table 7.12). The trend suggest that NHI values increased with increasing N rate. The NHI index values varied between 0.52 and 0.68 from the control treatment and soil applied urea at 60 kg N ha^{-1}

respectively. Nitrogen harvest index is closely associated with grain yield and this trend could be attributed to the responses observed for grain yield in Chapter 6 of this thesis. Rattunde and Frey (1986), and Kairudin and Frey (1988) reported that NHI responses were positively associated with grain yield in oats.

The effect of the interaction between N rate and the method of N application significantly ($p \leq 0.05$) influenced NHI in 2016. The NHI values ranged between 0.86 from liquid N applied at 60 kg ha^{-1} to 0.74 from soil applications at 60 kg N ha^{-1} (Table 7.13). The high NHI observed with liquid N at 60 kg ha^{-1} could be ascribed to increased translocation and partitioning of N to the grain (Bulman and Smith 1994). Plants that were sprayed with N probably accumulated high amounts of N before anthesis (Austin et al. 1977), which was later remobilized from storage organs into grain.

Applications of higher N rate probably increased the uptake of N compared to lower N rates although the increases were not statistically significant ($p \leq 0.05$) (Table 7.14). The effect of method of N applications showed that NHI was significantly ($p \leq 0.05$) affected by liquid applied N compared to granular soil N applications in 2016 only but not in 2013 and 2014 (Table 7.15). Middleton and Smith (1979) and Castle et al. (2006) found that less energy was required to synthesize protein from ammonium ions when N was sprayed on leaves than through roots where N must be converted to nitrate before uptake. This could probably be the contributing factor to the different responses demonstrated by these plants in this study.

7.3.2.3 Partial factor productivity (PFP_N)

The PFP_N was significantly ($p \leq 0.05$) affected by N treatments throughout the three years of this study (Table 7.10). The responses of PFP_N to the interaction between N rate and the method of N applications were significant in 2016 (Table 7.13). The results showed that granular N applications at 30 kg ha^{-1} produced a significantly higher PFP_N compared to granular applications at 60 kg ha^{-1}. Although the results showed that the two rates (30 and 60 kg N ha^{-1}) of liquid N application did not differ significantly with granular N applications at 30 kg N ha^{-1}, the trend tended to favour granular N applications at 30 kg N ha^{-1} and to a lesser extend liquid N at 30 kg ha^{-1}. Partial factor productivity is strongly influenced by N rate (Dobermann 2007) and several other authors have confirmed decreases of PFP_N with increasing N rates (Amanullah and Alams 2009; Amado et al. 2013; Panayatova and Kostadinova 2015). The reduced responses to liquid N application rates at 30 kg N ha^{-1} compared to granular N application could be ascribed to the imbalance in nitrogen use, N losses through leaching or reduction in the volume of roots (Karim and Ramasamy 2000).

Although the tendency showed that there was a strong effect of N application rate with lower N application rates resulting in higher PFP_N, the responses suggest that liquid N applications generally showed a superior performance compared to granular N applications (Table 7.17 and Table 7.18). The PFP_N varied between 32 g g^{-1} (liquid urea at 60 kg ha^{-1}) and 93 g g^{-1} (liquid urea at 30 kg ha^{-1}) during the three-year study period (Table 7.16). Extracting data sets to evaluate the effect of the method of N application confirmed that there were significant differences ($p \leq 0.05$) between the granular applied N and liquid N applications at least in two of the three study years (Table 7.18). This could be due to the ability of plants sprayed with liquid N sources to absorb and utilize the applied N more efficiently compared to granular soil applied N. Studies by Sabir et al. (2002) revealed that applications of N through foliage significantly increased yield components compared to granular soil applications of N in wheat. Mudaliar (1959) found that foliar application of N resulted into quicker N absorption compared to soil applications. Similarly, Bowman (1992) showed that foliar applied urea concentration in new and old leaves of perennial ryegrass turf reached maximum after 12 to 24 hours followed by a slow decline thereafter. The author attributed the high levels of NH_4^+ in the 12-hour samples to a very rapid hydrolysis of absorbed urea by leaves. As it was observed in Section A above, the effect of N rate showed that increasing N topdressing rate resulted into a decline in mean PFP_N. This study confirms the findings of several other authors on the effect of N rate on PFP_N responses (Amado et al. 2013; Dobermann 2007; Amanullah and Almas 2009). It seems that there is a consensus that higher PFP_N responses are superior with lower N rates compared to higher N rates (Ladha et al. 2005).

Table 7.12: Effect of N treatment on grain N uptake, straw N uptake, total N uptake and NHI under controlled glasshouse conditions in Glasshouse Experiment 1

	Grain N uptake			**Straw N uptake**			**N Total uptake**			**NHI**		
	(mg plant^{-1})											
Treatment	2013	2014	2016	2013	2014	2016	2013	2014	2016	2013	2014	2016
Control	20.47f	26.33g	47.80bcd	4.36f	24.70e	17.97ab	24.82e	51.03d	65.77bcd	0.82	0.52d	0.73
LAN 30+	38.23de	60.19de	58.44bc	16.25bc	39.32cd	16.76ab	54.48d	99.51bc	75.21bc	0.70	0.60bc	0.78
Granular LAN 30	37.52e	64.01cde	46.43cd	14.70de	41.09bcd	16.31ab	52.22d	105.10b	62.75cd	0.72	0.61bc	0.74
Granular LAN 60	54.21b	75.42b	59.44b	20.43bc	51.07ab	18.54a	74.63bc	126.49a	77.98ab	0.73	0.60bc	0.76
Granular Urea 30	40.45de	51.51f	49.72bcd	13.64	37.15cd	14.09b	54.09d	88.66c	63.81bcd	0.75	0.58bcd	0.78
Granular Urea 60	55.56b	69.26bc	41.48de	19.56cd	33.25de	16.36ab	75.11b	102.51b	57.85d	0.74	0.68a	0.72
Liquid Urea 30	49.16c	68.26bcd	32.42e	19.58cd	39.94cd	7.72c	68.74c	108.20b	40.14e	0.72	0.63abc	0.81
Liquid Urea 60	66.67a	73.10b	41.76de	29.61a	51.28a	9.99c	96.27a	124.38a	51.75de	0.69	0.59bcd	0.81
Liquid UAN 30	42.27d	56.50ef	58.50bc	13.97e	43.79abc	17.04ab	56.24d	100.29bc	75.54bc	0.75	0.56cd	0.77
Liquid UAN 60	68.19a	84.80a	82.42a	25.08ab	46.51abc	9.56c	93.28a	131.30a	91.98a	0.73	0.65ab	0.90
Mean	**47.27**	**62.94**	**51.84**	**17.72**	**40.81**	**14.44**	**64.99**	**103.75**	**66.28**	**0.73**	**0.60**	**0.78**
Cv (%)	**30.74**	**25.54**	**26.64**	**39.02**	**19.83**	**27.08**	**32.73**	**22.17**	**22.19**	**4.85**	**7.50**	**6.68**
P value	***0.005***	***0.007***	***0.010***	***0.010***	***0.010***	***0.031***	***0.007***	***0.010***	***0.010***	***0.060***	***0.010***	***0.693***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

LAN 30+ = LAN applied at 30 kg N ha^{-1} followed by foliar application of Activate N

Table 7.13: Effect of the interaction between N rate and method of N application on grain N uptake, total N uptake, nitrogen harvest index (NHI) and partial factor productivity (PFP_N) under controlled glasshouse conditions in Glasshouse Experiment 1

		Grain N uptake mg plant^{-1}			**Total N uptake mg plant^{-1}**			**NHI**			**PFP_N (g g^{-1})**		
N rate (kg ha^{-1})	**Method**	2013	2014	2016	2013	2014	2016	2013	2014	2016	2013	2014	2016
30	Granular	39.00d	57.67	48.00	53.00d	97.00	63.33	0.73	0.59	0.75bc	48.10	77.78	57.18a
30	Liquid	45.67c	62.33	45.33	62.33c	104.33	58.00	0.73	0.60	0.79b	56.71	90.28	49.91ab
60	Granular	55.00b	70.00	50.33	75.00b	115.00	68.00	0.73	0.64	0.74c	45.19	64.97	38.06b
60	Liquid	67.33a	79.00	62.00	94.67a	128.00	72.00	0.72	0.62	0.86a	54.07	66.67	48.95ab
Mean		**51.75**	**67.25**	**51.42**	**71.25**	**111.08**	**65.33**	**0.73**	**0.61**	**0.79**	**51.02**	**74.93**	**48.53**
Cv (%)		**23.74**	**13.88**	**14.28**	**25.30**	**12.13**	**9.24**	**0.07**	**3.60**	**6.92**	**10.38**	**15.62**	**16.24**
P Value		***0.035***	***0.719***	***0.152***	***0.008***	***0.463***	***0.398***	***0.584***	***0.523***	***0.01***	***0.924***	***0.055***	***0.045***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

Table 7.14: Effect of N rate on grain N uptake, straw N uptake, total N uptake, and NHI under controlled glasshouse conditions in Glasshouse Experiment 1

	Grain N uptake (mg plant^{-1})			Straw N uptake (mg plant^{-1})			Total N uptake (mg plant^{-1})			NHI		
N Rate (kg ha^{-1})	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**
30	42.35b	60.07b	46.77	15.47b	40.49	13.79	57.82b	100.56b	60.56	0.73	0.60	0.77
60	61.16a	75.64a	56.28	23.67a	45.53	13.61	84.82a	121.17a	69.89	0.72	0.63	0.80
Mean	**51.75**	**67.86**	**51.52**	**19.57**	**430.01**	**13.70**	**71.32**	**110.87**	**65.22**	**0.73**	**0.61**	**0.78**
Cv (%)	**25.69**	**16.22**	**13.05**	**29.61**	**8.28**	**0.91**	**26.77**	**13.14**	**10.12**	**0.98**	**3.45**	**2.7**
P Value	***0.000***	***0.000***	***0.058***	***0.000***	***0.083***	***0.884***	***0.000***	***0.000***	**0.096**	**0.484**	**0.087**	**0.126**

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

Table 7.15: Effect of N method of application on grain N uptake, straw N uptake, total N uptake, and NHI under controlled glasshouse conditions in Glasshouse Experiment 1

	Grain N uptake (mg g^{-1})			**Straw N uptake (mg g^{-1})**			**Total N uptake (mg g^{-1})**			**NHI**		
Method	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**	**2013**	**2014**	**2016**
Granular	46.93b	65.05b	49.27	17.08b	40.64	13.79	64.01b	105.69b	65.6	0.73	0.62	0.75b
Liquid	56.57a	70.66a	53.77	22.06a	45.38	13.61	78.63a	116.04a	64.85	0.72	0.61	0.82a
Mean	**51.75**	**67.86**	**51.52**	**19.57**	**43.01**	**13.70**	**71.32**	**110.87**	**65.22**	**0.73**	**0.61**	**0.78**
Cv (%)	**13.16**	**5.85**	**6.18**	**18**	**7.79**	**0.91**	**14.49**	**6.6**	**0.81**	**0.98**	**1.15**	**6.31**
P Value	***0.000***	***0.047***	***0.362***	***0.001***	***0.102***	***0.884***	***0.000***	***0.014***	***0.893***	***0.573***	***0.671***	***0.000***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

Table 7.16: Effect of N treatment on partial factor productivity (PFP_N under controlled glasshouse conditions in Glasshouse Experiment 1

	PFP_N ($g\ g^{-1}$)		
Treatment	**2013**	**2014**	**2016**
LAN 30+	48.43cde	82.87b	36.53c
Granular LAN 30	46.30ef	85.65ab	53.80ab
Granular LAN 60	43.46f	65.43cd	43.27bc
Granular Urea 30	49.91cde	69.91cd	60.56a
Granular Urea 60	46.91fdef	64.51cd	32.84c
Liquid Urea 30	61.94a	93.98a	36.67c
Liquid Urea 60	52.78bc	61.73d	32.65c
Liquid UAN 30	51.48bcd	86.57ab	63.15a
Liquid UAN 60	55.37b	71.60c	65.25a
Mean	**50.73**	**75.81**	**47.19**
Cv (%)	**10.91**	**15.32**	**28.63**
P value	***0.008***	***0.010***	***0.048***

Values within a column differ significantly at *p = 0.05* if they are followed by different letters

Table 7.17: Effect of N rate on partial factor productivity (PFP_N) under controlled glasshouse conditions in Glasshouse Experiment 1

	PFP_N ($g\ g^{-1}$)		
N Rate ($kg\ ha^{-1}$)	**2013**	**2014**	**2016**
30	52.41a	84.03a	53.54a
60	49.63b	65.82b	43.50b
Mean	**51.02**	**74.92**	**48.52**
Cv (%)	**3.85**	**17.18**	**14.63**
P Value	***0.064***	***0.000***	***0.027***

Values within a column differ significantly at *p = 0.05* if they are followed by different letters

Table 7.18: Effect of N treatment on partial factor productivity (PFP_N under controlled glasshouse conditions in Glasshouse Experiment 1

	PFP_N (g g^{-1})		
N Method	**2013**	**2014**	**2016**
Granular	46.64b	71.37b	47.62
Liquid	55.39a	78.47a	49.43
Mean	**51.02**	**74.92**	**48.52**
Cv (%)	**12.13**	**6.70**	**2.64**
P Value	***0.000***	***0.013***	***0.682***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

7.3.2.4 Multiple regression analysis

Table 7.20 shows the multiple regression analysis of different N use efficiency parameters with grain yield identified as the dependant variable. Due to severe multi-collinearity that existed among the different predictor variables straw N, total N uptake and NHI were excluded from the multiple regression analysis as shown in Table 7.20. Grain yield was significantly influenced by PFP_N and grain N uptake which accounted for 97% of the variation (Adjusted R^2=0.97). This analysis suggest that, if PFP_N is kept constant, a one unit increase in grain N uptake will result into 82% (b* = 0.82) unit increase in grain yield. If grain N uptake is held constant, a one-unit increase in PFP_N would increase grain yield by 22% (b* = 0.22) units.

Table 7.19: Multiple regression analysis of partial factor productivity (PFP_N), grain N uptake, straw N uptake, total N uptake and NHI on grain yield in Glasshouse Experiment 1

	Regression Summary for Dependent Variable: Grain Yield R=. 985007 R^2= .970238 Adjusted R^2= .969160						
	b*	Std.Err.	b	Std.Err.	t(116)	p-value	# times in best 20 models
Intercept			-1.148182	0.267148	-5.54681	0.000000	
PFP_N	0.223647	0.022874	0.04999	0.005113	9.77737	0.000000	5
Grain N Uptake	0.818115	0.022874	0.06671	0.001865	35.76629	0.000000	5
Straw N uptake	Excluded						
Total N Uptake	Excluded						5
NHI	Excluded						5

7.3.3 SECTION C - GLASSHOUSE EXPERIMENT 2

The Anova analysis in Table 7.20 below shows that the different N use efficiency parameters were significantly ($p \leq 0.05$) affected by N treatment in both 2014 and 2016.

Table 7.21 shows the Anova analyses of the effect of N rate, N timing, N method of application and associated interactions. This study showed that N rate significantly affected the different NUE parameters with the exception of NHI in 2014. No plant responses were observed due to timing of N application. The timing x method interaction showed a strong effect on different NUE parameters in 2014 but not in 2016 while the timing x rate interaction showed only significant effects in terms of PFP_N and NHI in 2014.

Table 7.20: Anova analysis of variance (ANOVA) for PFP_N, grain N uptake, straw N uptake, total N uptake and NHI under glasshouse conditions in Glasshouse Experiment 2

	N treatment	
Factor	**2014**	**2016**
PFP_N	*	*
Straw N uptake	*	*
Grain N uptake	*	*
Total N uptake	*	*
NHI	*	*

* Significant at the 0.05 probability level

ns = not significant at the 0.05 probability level

Table 7.21: Anova analysis of variance (ANOVA) for the N timing of N application, N rate, method of N application on PFP_N, AE, straw N, grain N, total N, NHI and ANR under glasshouse conditions in Glasshouse Experiment 2

	Timing (T)		**N Rate (R)**		**Method (M)**		**TxR**		**TxM**		**RxM**		**TxRxM**	
Factor	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**
PFP_N	ns	ns	*	ns	ns	ns		ns	*	ns	ns	ns	ns	ns
Straw N uptake	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Grain N uptake	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Total N uptake	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
NHI	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level

ns = not significant at the 0.05 probability level

7.3.3.1 Grain N uptake, straw N uptake and total N uptake

The effect of treatment on grain N uptake, straw N uptake and total N uptake is shown in Table 7.22. There did not appear to be significant differences between split applications and single applications for N uptake. The trends however, suggest that grain N uptake was generally improved through the split applications for soil applied granular N. In contrast, the responses demonstrated by liquid N applications show that the single application resulted in a better response compared to split applications. Similarly, these trends were observed for straw N uptake and total N effect as would be expected. The effect was rather strong in 2014 compared to 2016. The effect of split applied liquid N could be due to N shortages during the period between tillering and anthesis because of low N uptake. Palta et al. (1994) mentioned that most of N taken up by the plants is accumulated between tillering and stem elongation. Arduini et al. (2009) revealed that N shortages during this period and subsequent shoot development, lead to higher shoot mortalities, smaller spike size, and limited final number of kernels produced per unit area.

Topdressing with granular N once at tillering probably resulted into losses of N through leaching compared to split applications. Velasco et al. (2012) reported that applications of N early in the season increases the risk of N loss from the root zone through leaching and denitrification. These authors found that splitting N resulted into significant above-ground N uptake (straw + grain) increases compared to single applications early in the season in wheat. This was strongly associated with a high crop capacity to assimilate N during the development stages closer to anthesis (Wuest and Cassman 1992).

The effect of N rate showed that increasing N topdressing rate resulted into an increase in grain N uptake, straw N uptake, and total N uptake where the effect was significant (Table 7.23). Chen et al. (2016) also reported increases of N uptake with increasing N application rate in wheat studies conducted in China. The responses could probably be an indication that the available N at lower N topdressing rates was partitioned towards grain yield production, which competed with grain N uptake (Tran and Tremblay 2000).

The effect of the interaction between method of application and timing is shown in Table 7.24.

The trends on N uptake showed that split applications of N for granular N sources and a single liquid N application were superior compared to the other interactions. These results show similar patterns observed for different parameters in Chapter 6 when this interaction was evaluated. These responses demonstrate a strong contrasting effect between granualr and liquid applied N fertilisers in this study. As was already mentioned above, N applied, as a granular source early in the season is vulnerable to different N loss pathways (Cassman et al. 2002) compared to split applications, which could explain the difference in plant responses. On the one hand, the observations on liquid N applications tend to favour single compared to split applications. This could be an expression of relatively less N uptake and translocation efficiency at lower liquid N rates compromising the effect of split applications compared to single liquid N applications.

7.3.3.2 Nitrogen harvest index (NHI)

The effect of N treatment significantly ($p \leq 0.05$) affected NHI in both 2014 and 2016 (Table 7.22). To a certain extent, the responses followed a similar pattern as was shown in N uptake illustrating the existing relationship between these N use parameters. Most importantly, the trends tended to favour higher N applications in 2016 compared to lower N rates (Table 7.23). According to Fischer (1993), NHI indicates the allocation efficiency of N into grains and it would be expected that conditions that enhance grain N uptake would produce higher NHIs. In contrast, Daba (2017) recorded higher NHI at the lowest N rate although this was not significantly different to higher N rate. The author attributed this to partitioning of total N content more to the vegetative plant components than to the grain, increasing the total aboveground biomass yield.

The interaction between the method of N applications and timing suggest that there was a close relationship between grain N uptake and NHI where the effect was significant as can be seen in Table 7.24. In 2014, a higher NHI (0.62) was achieved when N was either split applied as granular N application or applied once at tillering as a liquid spray.

Table 7.22: Effect of N treatment on grain N uptake, straw N uptake, total N uptake and NHI under glasshouse conditions in Glasshouse Experiment 2

	Grain N uptake (mg plant^{-1})		Straw N uptake (mg plant^{-1})		N Total uptake (mg plant^{-1})		NHI	
Treatment	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**
Control	17.04e	44.15cd	17.45e	9.55abc	34.49f	53.70cde	0.49e	0.82def
Granular Urea 30T	37.81cd	47.41bcd	27.33bc	5.36e	65.14de	52.77de	0.58bcd	0.90abc
Granular Urea 15TF	46.53abc	53.53abc	29.55abc	10.08ab	76.08abcd	63.61abcd	0.61abcd	0.84de
Granular Urea 60T	42.54bcd	65.99a	31.59a	5.83de	74.13abcd	71.81ab	0.57cd	0.92ab
Granular Urea 30TF	50.20ab	65.80a	29.04abc	11.55a	79.24abc	77.35a	0.63ab	0.85cde
Liquid Urea 30T	43.27bcd	39.07cd	29.13abc	11.00ab	72.40bcde	50.07de	0.60bcd	0.78f
Liquid Urea 15TF	37.72cd	34.88d	30.71abc	5.23ef	68.43cde	40.10e	0.55d	0.87bcd
Liquid Urea 60T	54.51a	43.94cd	31.92a	8.54bcd	86.43a	52.48de	0.63ab	0.84de
Liquid Urea 30TF	45.01abc	49.00bcd	29.77ab	10.07ab	74.78abcd	59.07bcd	0.60bcd	0.83def
Liquid UAN 30T	42.69bcd	49.07bcd	21.21abc	7.23cde	63.90de	56.29cd	0.67a	0.87bcd
Liquid UAN 15TF	33.26d	45.16cd	26.95d	11.60a	60.21e	56.76bcd	0.55d	0.80ef
Liquid UAN 60T	51.33ab	46.64cd	32.12c	2.52f	83.45ab	49.16de	0.62abc	0.95a
Liquid UAN 30TF	45.74abc	61.72ab	27.09a	7.14cde	72.83bcde	68.86abc	0.63ab	0.90abc
Mean	**42.13**	**49.72**	**27.99c**	**8.13**	**70.12**	**57.85**	**0.60**	**0.86**
Cv (%)	**22.64**	**19.4**	**15.31**	**34.85**	**18.63**	**17.7**	**7.85**	**5.67**
P Value	***0.000***	***0.001***	***0.010***	***0.025***	***0.000***	***0.010***	***0.010***	***0.007***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

UAN = Urea ammonium nitrate, T = applied at tillering, TF = applied at tillering and flowering

Table 7.23: Effect of N rate on grain N uptake, straw N uptake, total N uptake and NHI under glasshouse conditions in Glasshouse Experiment 2

Effect	**Grain N uptake**		**Straw N uptake** (mg plant^{-1})		**Total N uptake**		**NHI**	
N Rate (kg ha^{-1})	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**
30	40.70b	46.25b	27.72b	8.25	68.54b	54.50b	0.59	0.85b
60	47.78a	58.11a	30.03a	7.87	78.03a	65.99a	0.60	0.88a
Mean	**44.24**	**52.18**	**28.94**	**8.06**	**73.29**	**60.24**	**0.60**	**0.87**
Cv (%)	**11.31**	**16.06**	**5.64**	**3.27**	**9.15**	**13.49**	**1.19**	**2.45**
P Value	***0.004***	***0.000***	***0.008***	***0.651***	***0.001***	***0.002***	***0.163***	***0.043***

Values within a column differ significantly at $p= 0.05$ if they are followed by different letters

Table 7.24: Effect of the interaction between method of N application and timing on grain N uptake, straw N uptake, total N uptake and NHI under glasshouse conditions in Glasshouse Experiment 2

Effect		Grain N uptake		Straw N uptake		Total N uptake		NHI	
		(mg plant^{-1})							
Method	**Timing**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**	**2014**	**2016**
Granular	T	40.17b	56.67	25.45	5.59c	69.63ab	62.29	0.57b	0.91
Granular	TF	48.36a	59.67	29.30	10.81a	77.66a	70.47	0.62ba	0.84
Liquid	T	47.95a	44.68	28.59	7.32c	76.55a	52.00	0.62ba	0.86
Liquid	TF	40.43b	47.69	28.63	8.41b	69.06bc	56.20	0.58b	0.85
Mean		**44.23**	**52.18**	**28.99**	**12.28**	**73.22**	**60.24**	**0.60**	**0.86**
Cv (%)		**10.26**	**13.67**	**1.54**	**57.21**	**6.15**	**13.32**	**4.40**	**3.59**
P Value		***0.001***	***0.994***	***0.916***	***0.015***	***0.008***	***0.567***	***0.003***	***0.082***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

T = applied at tillering, TF = applied at tillering and flowering

7.3.3.4 Partial factor productivity (PFP_N)

The PFP_N was significantly ($p \leq 0.05$) affected by N treatments in both years of this study. Table 7.25 shows that PFP_N varied between 53.89 (granular urea 60) and 85 g g^{-1} (granular urea 15TF) in 2014 and between 31.67 (liquid urea 30TF) and 56.5 g g^{-1} (granular urea 15TF) in 2016. The PFP_N values were consistent and strongly influenced by the total amount of N applied irrespective of the method of application. The effect of N rate shows that PFP_N values decreased with increasing N rates as was demonstrated in Section A and B above (Table 7.26). These results are confirming the observation reported in the two sections above on PFP_N. Amanullah and Almas (2009) reported that PFP_N showed negative relationship with increasing N rates for wheat in wheat-maize cropping systems in Pakistan. The decline in PFP_N could be due to nutrient imbalance, low soil N mineralization or reduced root volume (Karim and Ramasamy 2000). Muchow (1998) and Halvorson et al. (2005) reported that NUE generally decrease with increasing level of available N rate.

The interaction between the method of application and time of N application showed that higher PFP_N values were produced following the application of liquid N once at tillering or granular N applied twice at tillering and early anthesis (Table 7.27). Again, the plant responses to this interaction were consistent for the different NUE parameters highlighting the inter-relationships existing between these NUE indices.

Table 7.25: Effect of N treatment on partial factor productivity (PFP_N) under glasshouse conditions in Glasshouse Experiment 2

	PFP_N ($g\ g^{-1}$)	
Treatment	**2014**	**2016**
Granular Urea 30T	74.83ab	55.83a
Granular Urea 15TF	85.00a	56.50a
Granular Urea 60T	53.89d	44.78abc
Granular Urea 30TF	64.44bcd	44.33abc
Liquid Urea 30T	83.33a	48.00ab
Liquid Urea 15TF	71.67abc	35.00cd
Liquid Urea 60T	65.56bcd	33.67cd
Liquid Urea 30TF	57.78bcd	31.67d
Liquid UAN 30T	83.33a	50.67ab
Liquid UAN 15TF	65.83bcd	45.00abc
Liquid UAN 60T	63.33bcd	35.44cd
Liquid UAN 30TF	55.00cd	41.00bcd
Mean	**68.67**	**43.49**
Cv (%)	**16.02**	**19.33**
P Value	***0.010***	***0.010***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

UAN = Urea ammonium nitrate, T = applied at tillering, TF = applied at tillering and flowering

Table 7.26: Effect of N rate on partial factor productivity (PFP_N) under glasshouse conditions in Glasshouse Experiment 2

Effect	**PFP_N ($g\ g^{-1}$)**	
N rate ($kg\ ha^{-1}$)	**2014**	**2016**
30	77.98a	50.42a
60	59.79b	40.00b
Mean	**68.89**	**45.21**
CV (%)	**18.67**	**16.30**
P Value	***0.000***	***0.000***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

Table 7.27: Effect of method of N application and timing on partial factor productivity (PFP_N) under glasshouse conditions in Glasshouse Experiment 2

Effect		PFP_N ($g\ g^{-1}$)	
Method	**Timing**	**2014**	**2016**
Granular	T	64.36ab	50.31
Granular	TF	74.72a	50.42
Liquid	T	73.89a	41.94
Liquid	TF	62.57b	38.17
Mean		**68.89**	**45.21**
CV (%)		**9.16**	**13.6**
P Value		***0.004***	***0.469***

Values within a column differ significantly at $p = 0.05$ if they are followed by different letters

T = Tillering, TF = Tillering and Flowering

7.3.3.5 Multiple regression analysis

The multiple regression analysis of different N use efficiency parameters for this experiment is shown in Table 7.28. Grain N uptake, straw N uptake and NHI were excluded from the analysis due to severe multi-collinearity. Grain yield was significantly influenced by PFP_N and total N uptake, which explained 91% of the variation (Adjusted R^2=0.91). In this model, if PFP_N is held constant, a one unit increase in total N uptake will result in 68% (b* = 0.68) unit increase in grain yield. On the other hand, if total N uptake is constant, a 35% (b* =0.35) unit increase would be achieved through a one unit increase in PFP_N .

Table 7.28: Multiple regression analysis of PFP, Grain N uptake, straw N uptake, total N uptake and NHI, on grain yield in Glasshouse Experiment 2

	Regression Summary for Dependent Variable: Grain Yield R=. 956165 R^2= .914251 Adjusted R^2= .912033						
	b*	Std.Err.	b	Std.Err.	t(116)	p-value	# times in best 20 models
Intercept			-2.19275	0.33704	-5.50523	0.000000	
PFP_N	0.352206	0.038426	0.04911	0.005358	9.16593	0.000000	5
Total N Uptake	0.681277	0.0038426	0.03883	0.002190	17.72977	0.000000	5
Straw N uptake	Excluded						5
Grain N Uptake	Excluded						5
NHI	Excluded						5

7.4 Conclusions

This study revealed that PFP_N decreased with increasing N rate irrespective of the method of application for both field and glasshouse studies. Plant N uptake (straw + grain) generally increased with increasing N application rate where the effect was significant throughout this study. The interaction between the method of N application and N rate showed that PFP_N was significantly improved by liquid N applications at 30 kg N ha^{-1} compared to other interactions in 2014 at Roodebloem and no significant interaction effect was observed in 2015. A similar trend was observed for this interaction for NUtE at this locality in 2014 but liquid N at 30 kg ha^{-1} did not differ significantly with granular applied N at 60 kg ha^{-1}. No method x N rate interaction was observed for NUtE and PFP_N at Langgewens in 2015 and this was probably due to poor soil moisture conditions because of seasonal drought. The multiple regression analysis demonstrated that grain N uptake and PFP_N were the most influential NUE indices that contributed to grain yield as these two variables accounted for the larger component of the variation for both Roodebloem and Langgewens.

The first glasshouse experiment (SECTION B) showed that liquid N applications significantly improved the different studied NUE parameters compared to granular N applications. The higher NUE efficiencies of liquid N applications could be due to less selective ability of the stomata to absorb the solutes through diffusion, which is not the case with granular N applications. The multiple linear regression analysis confirmed that PFP_N and grain N uptake were the most two significant input variables for the improvement of grain yield in this study as was observed for the field studies.

The second glasshouse experiment revealed that the different NUE parameters were more favoured by the liquid N applications at 30 kg N ha^{-1} although this did not differ significantly with 60 kg N ha^{-1} of granular N applications where the effect was significant. This interaction (method x N rate) was consistent throughout this study and proved that benefits in the improvement of NUE could be achieved through the split applications of granular applied N, while the liquid applications were more superior with the single application.

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Chapter 8

Summary and general conclusions

Changes in modern agriculture have put emphasis on environmental friendly and sustainable agricultural production practices in recent years. Wheat (*Triticum aestivum* L.) is regarded as one of the most significant and valuable food crops for the world human population (FAOSTAT 2010). At the same time, nitrogen (N) is one of the most important nutrient elements that affect both grain yield and grain protein content of wheat. Several authors (Cassman et al. 2002; Dobermann 2005, 2007; Ladha et al. 2005) have illustrated significant improvements in the efficiency with which this element is utilized by the plants. However, results still show that a significant proportion of the applied N is not recovered by the crop, resulting into low N use efficiencies (Gupta and Khosla 2012), environmental pollution and increased costs of production (Sharpe et al. 1988; Semenov et al. 2007). The poor N use efficiencies are attributed to various N losses through gaseous plant emmission (Harper et al. 1987; Francis et al. 1993), soil denitrification (De Datta et al. 1991; Hilton et al. 1994), surface runoff (Blevins et al. 1996), volatilization (Hargrove et al. 1977), and leaching (Olson and Swallow 1984; Raun and Johnson 1995). Amongst other technologies used in improving N use efficiencies, liquid applications of N to wheat have demonstrated better plant responses (Strong 1982; Bly and Woodard 2003; Amanullah et al 2015).

Although there are reports of incorporating liquid (sprays) applications of N in wheat in South Africa (Laubscher 1981; Du Plessis and Agenbag 2004), there is a lack of information on use of liquid N topdressings compared to the rest of the world on this crop under local conditions especially on N use efficiencies. A study was therefore conducted to evaluate the effect of granular (LAN, granular urea) and liquid (UAN and liquid urea) N topdressings on N use efficiencies, grain yields, yield and quality parameters of spring wheat under field and glasshouse conditions from 2013 to 2016.

Specific objectives of the study and the results obtained were as follows:

Effect of granular and liquid applied N topdressings on grain yield,yield and quality parameters of wheat under field conditions

The study revealed that the effect of N fertiliser topdressing treatment was not consistent on studied parameters over the three-year period. These inconsistencies were associated with total seasonal rainfall and rainfall distribution, which influenced the soil N mineralization and soil N losses through leaching. Furthermore, the effect of ryegrass and lower rainfall (drought conditions) in 2014 and 2015 (respectively) at Langgewens contributed to these inconsistencies. Although not significant, the effect of method of N application showed that liquid N applications produced relatively higher grain yield compared to granular N topdressing for grain yields in four of the six site years (2013 and 2014 at both localities) of this study.

The effect of N application rate showed that grain yields significantly (*$p \leq 0.05$*) increased with increasing N level at Langgewens in the 2013 growing season. The interactions between N rate and method of application showed that there was a significantly (*$p \leq 0.05$*) better grain yield when N was applied as liquid N at 30 kg ha^{-1} compared to granular N at 30 kg N ha^{-1} and liquid N at 60 kg N ha^{-1}. This significant interaction confirmed and strengthened the argument of other workers who reported significant improvements in yields following liquid N (sprays) topdressing after soil N applications at sowing.

The effect of locality showed that Roodebloem produced significantly (*$p \leq 0.05$*) higher grain yields compared to Langgewens. The lower yield responses at Langgewens were associated with resistant ryegrass that competed with wheat in 2014 and lower seasonal rainfall in 2015. There was no clear trend observed for 1000-kernel mass, hectolitre mass, plant biomass and harvest index due to the effect of N fertiliser treatment for both localities. Thousand kernel mass and the hectolitre mass varied largely due to season and locality as a result of variation in seasonal rainfall. The analysis of the interaction between season and locality for water use efficiency (WUE) showed significant responses and the tendency favoured Roodebloem compared to Langgewens although Langgewens showed a significantly better water use efficiency response in 2013. The N fertiliser treatment did not significantly influence grain protein content (GPC) and falling number (FN) although the trend tended to favour 60 kg N ha^{-1} topdressing over 30 kg N ha^{-1} for grain protein content. The multiple linear regression analysis showed that WUE and to a lesser extent, HLM significantly influenced grain yield for the two localities.

Effect of granular and liquid applied N topdressing on yield and grain quality parameters under glasshouse conditions

Studies conducted under glasshouse conditions showed that liquid application of UAN at 60 kg N ha^{-1} produced significantly ($p \leq 0.05$) better results and was consistent in all the years for grain yield. The effect of N application rate significantly affected plant biomass, number of ears, mass of ears, grain yield and to a lesser extent, the harvest index. This showed that applications of 60 kg N ha^{-1} significantly ($p \leq 0.05$) improved grain yields and yield parameters compared to applications at 30 kg N ha^{-1}. The method of application showed that liquid applied N significantly increased the measured parameters compared to granular applied N throughout the study. The effect of the interaction between N rate and method of application did not show any effect with the exception of grain yield and harvest index in 2016. This interaction showed that significantly ($p \leq 0.05$) higher grain yield was obtained following liquid N topdressing at 60 kg N ha^{-1} compared to other interactions. The N fertiliser treatment did not show any significant effect on GPC and FN. The multiple linear regression analysis showed that plant biomass and harvest index significantly influenced grain yield. It can be concluded that the application of N to the soil at sowing combined with liquid N topdressings applied at tillering was beneficial to wheat in terms of grain yields and yield parameters in this study.

Effect of single and split N fertiliser topdressings on grain yield and quality parameters under glasshouse conditions

The results of this study showed that the different growth parameters were significantly ($p \leq 0.05$) affected by the N treatment. The effect of higher N rate resulted into significantly better plant growth and development, and ultimately higher grain yields and yield parameters. The effect of the method of application tended to favour granular N topdressings where the effect was significant. The interaction between the method of N application and time of N application showed that liquid N topdressings at tillering promoted significantly ($p \leq 0.05$) higher responses compared to liquid N split applications (tillering and flowering) although the effect did not differ significantly to the two levels of granular N applications. This interaction also showed that split applications of granular applied N generated relatively better grain yield responses compared to single granular N applications, although the effect lacked significance. This interaction indicated that single liquid N topdressing early in the season could be preferred where the objective is to improve grain yield compared to split applications of liquid N topdressings. In contrast, the synchronisation between N availability and N demand was illustrated by the relatively better responses from the split applications of granular N compared to single N applications for the different parameters although the differences were not statistically significant between these two applications timings. The multiple linear regression analysis showed that grain yield was significantly affected by mass of ears and to a lesser extent, harvest index. Grain protein content and the falling number was not affected by the N fertiliser treatment, although, the trends favoured split applied N over single topdressing for grain protein content. No clear trend was observed for the falling number.

Effect of granular and liquid N topdressing on nitrogen use efficiencies of spring wheat under field and glasshouse conditions

Studies (SECTION A in Chapter 7) on nitrogen use efficiency under field conditions at Roodebloem and Langgewens showed that partial factor productivity (PFP_N); expressed as grain yield divided by applied N decreased with increasing N rate irrespective of the method of application for both localities. Straw N uptake and grain N uptake increased with increasing N application rate where the effect was significant throughout the study. The interaction between the method of N application and N rate showed that PFP_N was significantly improved by liquid N applications at 30 kg N ha^{-1} compared to other interactions in 2014 at Roodebloem and no significant interaction effect was observed in 2015. These findings were in agreement with several other reports on PFP_N under similar and different ecological zones elsewhere in the world. Similar patterns were observed for the method of N application and N rate for N utilization efficiency (grain yield/total N uptake) at Roodebloem in 2014 but liquid N topdressings at 30 kg ha^{-1} did not differ significantly with granular N topdressings at 60 kg ha^{-1}. No method x N rate interaction was observed for nitrogen utilization efficiency and PFP_N at Langgewens in 2015 and this was strongly associated with low soil moisture conditions due to seasonal drought in 2015. The multiple regression analysis demonstrated that grain N uptake and PFP_N were the most influential NUE indices that contributed to grain yield as these two variables accounted for the larger component of the variation for both Roodebloem and Langgewens.

The first glasshouse experiment (SECTION B in Chapter 7) showed that liquid N topdressings significantly improved the different studied NUE parameters compared to granular N topdressings. The higher NUE efficiencies of liquid N applications were associated with less selective ability of the stomata to absorb the solutes through diffusion, which is not the case with granular (soil applied) N applications. The multiple linear regression analysis confirmed that PFP_N and grain N uptake were the two most significant input variables for the improvement of grain yield in this study as was observed for the field studies.

The second glasshouse experiment (SECTION C in Chapter 7) showed that the different NUE parameters were improved by the liquid N topdressings at 30 kg N ha^{-1} although this did not differ significantly with 60 kg N ha^{-1} of granular N topdressings. This interaction (method x N rate) was consistent throughout this study and proved that benefits in the improvement of

NUE could be achieved through the split applications of granular applied N, while the liquid N applications were more superior with the single N topdressing at tillering compared to split applications.

Overall, if the responses of grain yield that resulted from the interaction between the method of N application and N rate under field conditions at Roodebloem in 2014 are taken into account, it can be concluded that combinations of N applications at sowing and liquid N topdressing at tillering could be useful in improving grain yields in wheat. This was further supported by the responses to the method of N application, where liquid N topdressing in four out of six site years showed relatively better grain yield responses compared to granular N topdressings, although the effect was not significant. These conclusions were also supported by the evaluations of NUE, which showed better responses from liquid N topdressings for the studied parameters where the N treatment and interactive effects were significant. One glasshouse experiment similar to the field experiment confirmed that liquid N topdressings were more effective in promoting different studied parameters compared to granular N topressings. It can be concluded in this study that, combinations of soil N applications at sowing with liquid N topdressings at tillering improved N use efficiency, grain yield and yield parameters of spring wheat.

The areas that are recommended for further studies resulting from this study include but are not limited to:

(i) The effect of single and split granular and liquid applied N topdressings on grain yield and protein content under field conditions as this effect was not incorporated in the current study under field conditions. This is triggered by the lack of responses in grain quality parameters demonstrated by spring wheat in this study. This is of importance particularly for grain protein content, which plays a significant role in the marketability of grain. Other quality parameters such as the falling number and the 1000-kernel mass were generally in the acceptable ranges and did not warrant any cause for concern at least from the findings of this study both under field and glasshouse conditions.

(ii) Carefully structured experiments that will evaluate and possible distinguish the conditions through which the responses to liquid applied N are induced should also be considered. In this case, it would be beneficial to incorporate studies that would focus on the physiological processes of the crop in relation to different climatic factors, such as rainfall (soil moisture) and temperature (both soil and air) on soil N mineralization, plant N uptake and N losses as seasonal rainfall was identified as the major source of variation in the current study. These studies can be evaluated both under field and glasshouse conditions (soil moisture), as glasshouse conditions offer better opportunities to manage soil water and temperature regimes.

(iii) The effect of plant growth promoting *rhizobacteria* (PGPRs) on N mineralization, N uptake and other potential benefits should also be visited. This is triggered by the relatively better responses for grain yields and N utilization efficiency observed in this study. Trends in the modern agriculture require researchers to put emphasis on production practices that will reduce the effect of N on environmental pollution while still ensuring that the major cereal grains are produced under sustainable production practices.

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APPENDICES

APPENDIX 1: ANOVA for Chapter 4

ANOVA analysis for treatment effects at Roodebloem and Langgewens from 2013 to 2015

Rood 2013					Lang 2013				
Source	MS Effect	MS Error	F	p	Source	MS Effect	MS Error	F	p
GY	167256.2	71506.85	2.339024	0.690000	GY	62711.49	23299.92	2.691489	0.026846
DKM	1.992593	0.183422	10.86346	0.010000	DKM	2.053704	0.352734	5.822250	0.730000
HLM	0.310764	0.074074	4.195313	0.030000	HLM	0.157407	0.112875	1.394531	0.249123
Rood 2014					Lang 2014				
Source	MS Effect	MS Error	F	p	Source	MS Effect	MS Error	F	p
PB	879.5690	454.7609	1.934135	0.050000	PB	5006.053	2088.946	2.396449	0.042029
GY	30224.54	9473.444	3.190449	0.011135	GY	158597.7	58297.16	2.720504	0.610000
DKM	1.109764	0.242424	4.577778	0.001412	DKM	1.724675	0.367150	4.697471	0.010000
HLM	0.185212	0.038788	4.775000	0.110000	HLM	0.185212	0.038788	4.775000	0.100000
HI	0.000218	0.000084	2.595984	0.029818	HI	0.001454	0.000734	1.980682	0.080605
Rood 2015					Lang 2015				
Source	MS Effect	MS Error	F	p	Source	MS Effect	MS Error	F	p
PB	17928.25	10024.74	1.788400	0.115691	PB	4482.982	1257.765	3.564245	0.004897
Grain yield	13559.25	6834.338	1.983988	0.080106	GY	37725.48	29598.25	1.274585	0.298614
DKM	0.633189	0.154589	4.095942	0.500000	DKM	0.362482	0.695652	0.521068	0.869286
HLM	0.227802	0.180354	1.263080	0.304768	HLM	1.410823	0.483092	2.920403	0.110000
HI	0.060438	0.011631	5.196479	0.890000	HI	0.001325	0.000277	4.788592	0.290000

ANOVA analysis for N rate, method of N application and interactions at Roodebloem from 2013 to 2015

Rood 2013			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	0.928499	0.346760	0.238095	0.630892	0.692308	0.415205	-	-	-	-
N Rate	1	20	0.432112	0.518449	2.142857	0.158776	0.076923	0.784358	-	-	-	-
Method*N rate	1	20	2.434030	0.134412	0.238095	0.630892	0.692308	0.415205	-	-	-	-
Rood 2014			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	0.131047	0.721146	0.882353	0.358765	2.558140	0.125406	0.351801	0.559743	0.132013	0.720166
N Rate	1	20	0.922763	0.348221	0.882353	0.358765	1.712474	0.205504	0.225669	0.639900	1.617162	0.218078
Method*N rate	1	20	7.727401	0.011561	0.882353	0.358765	0.084567	0.774196	3.883840	0.080543	0.000000	1.000000
Rood 2015			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	0.018040	0.894498	0.000000	1.000000	2.195122	0.154033	0.380802	544136	0.296121	0.592340
N Rate	1	20	0.284604	0.599579	0.588235	0.452062	0.975610	0.335085	1.215886	0.283262	0.260797	0.615164
Method*N rate	1	20	0.343635	0.564297	2.352941	0.140715	0.000000	1.000000	0.26384	0.613227	0.168281	0.686005

ANOVA analysis for N rate, method of N application and interactions at Langgewens from 2013 to 2015

Lang 2013			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	5.412810	0.030612	2.142857	0.158776	0.285714	0.598873	-	-	-	-
N Rate	1	20	3.850125	0.063808	0.238095	0.630892	0.000000	1.000000	-	-	-	-
Method*N rate	1	20	0.647315	0.430535	0.238095	0.630892	0.285714	0.598873	-	-	-	-
Lang 2014			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	0.071899	0.791337	0.204409	0.656051	1.526480	0.230958	0.204409	0.656051	0.211268	0.650733
N Rate	1	20	0.097556	0.758016	0.411156	0.528664	0.124611	0.727780	0.411156	0.528664	0.414085	0.527214
Method*N rate	1	20	0.058381	0.811534	0.057098	0.813575	0.000000	1.000000	0.057098	0.813575	0.076056	0.785544
Lang 2015			GY		TKM		HLM		PB		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	20	1.289052	0.269646	1.623377	0.217229	0.643045	0.432036	0.683962	0.417984	0.250564	0.622142
N Rate	1	20	0.674110	0.421302	0.064935	0.801461	0.643045	0.432036	0.987705	0.332177	0.632044	0.435940
Method*N rate	1	20	0.000045	0.994712	0.064935	0.801461	0.118110	0.734680	0.026091	0.873299	0.195075	0.663464

ANOVA analysis for year, locality and interactions for Roodebloem and Langgewens from 2013 to 2015

			GY		DKM		HLM	
Effect	Num. DF	Den. DF	F	p	F	p	F	p
Year	2	168	95.2311	0.000000	276.2081	0.000000	128.5552	0.000000
Locality	1	168	230.4881	0.000000	7.5672	0.006596	337.5886	0.000000
Year*Locality	2	168	284.5170	0.000000	131.3212	0.000000	493.2367	0.000000
			PB		HI		WUE	
Effect	Num. DF	Den. DF	F	p	F	p	F	p
Year	2	168	336.0090	0.000000	4.9360	0.028875	3.6910	0.027547
Locality	1	168	7.3300	0.000000	35.7850	0.000000	338.5890	0.000000
Year*Locality	2	168	6.2120	0.014564	33.6150	0.000000	195.7950	0.000000

APPENDIX 2: ANOVA for Chapter 5

ANOVA analysis for treatment effects in Glasshouse Experiment 1

	2013				2014				2016			
Source	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p
PB	9.366255	2.104074	4.451485	0.010000	20.37249	10.28348	1.981090	0.010000	12.11957	5.310897	2.282020	0.031262
NEPP	0.402469	0.176296	2.282913	0.010000	0.769547	0.838148	0.918152	0.010000	0.423045	0.597037	0.708575	0.020000
MEPP	1.376963	0.306130	4.497974	0.000234	4.365409	2.368400	1.843189	0.010000	2.705653	2.189251	1.235881	0.010000
GY	0.624200	0.208370	2.995625	0.006212	2.189095	0.863426	2.535359	0.017611	2.073846	1.539441	1.347142	0.010000
HI	0.001460	0.001076	1.356811	0.050000	0.004607	0.001702	2.706825	0.011937	0.001393	0.001596	0.872793	0.010000

ANOVA analysis for N rate, method of N application and treatments in Glasshouse Experiment 1

2013			GY		PB		NEPP		MEPP		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	44	168.9888	0.000000	115.8467	0.000000	77.19006	0.000000	118.7804	0.000000	1.605775	0.211753
N Rate	1	44	38.3194	0.000000	22.4279	0.000023	5.34557	0.025516	46.2906	0.000000	0.018204	0.893289
Method*N rate	1	44	2.4368	0.125682	2.8032	0.101171	1.16415	0.286487	1.0480	0.311559	0.023156	0.879749
2014			GY		PB		NEPP		MEPP		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	44	18.95705	0.000079	4.830836	0.033262	7.564186	0.008609	13.52906	0.000636	3.099915	0.085246
N Rate	1	44	4.96581	0.031011	2.649077	0.110751	1.520033	0.224161	9.86447	0.003010	0.063264	0.802581
Method*N rate	1	44	2.17320	0.147553	0.081316	0.776861	0.440720	0.510239	0.94772	0.335623	2.105666	0.153847
2016			GY		PB		NEPP		MEPP		HI	
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
Method	1	44	4.449072	0.040645	2.096173	0.154757	0.057743	0.811216	5.469400	0.023959	3.553452	0.066036
N Rate	1	44	0.667545	0.418311	1.894972	0.175607	0.230971	0.633186	0.000139	0.990641	7.829567	0.007599
Method*N rate	1	44	4.519691	0.039155	0.092439	0.762532	0.923885	0.341710	3.729010	0.059934	9.203754	0.004043

APPENDIX 3: ANOVA for Chapter 6

ANOVA analysis for N rate, method, timing and interactions in Glasshouse Experiment 2

				PB		NEPP		MEPP		GY		HI	
2014	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
	Timing	1	52	1.27931	0.263217	0.139535	0.710262	0.131055	0.718808	0.001472	0.969546	0.667173	0.417766
	N level	1	52	10.37647	0.002203	8.201550	0.006022	7.770251	0.007400	7.262400	0.009461	0.888248	0.350311
	Method	1	52	0.13946	0.710339	0.558140	0.458376	0.011795	0.913934	0.052977	0.818864	0.098694	0.754659
	N level*Timing	1	52	0.28584	0.595180	0.992248	0.323806	0.131055	0.718808	0.220831	0.640372	2.600702	0.112870
	Method*Timing	1	52	6.12252	0.016647	8.201550	0.006022	6.059983	0.017181	9.184207	0.003798	5.211492	0.026556
	N level*Method	1	52	2.09695	0.153596	3.488372	0.067442	1.993346	0.163948	0.437891	0.511064	0.021493	0.884010
	N level*Method*Timing	1	52	0.13946	0.710339	0.992248	0.323806	0.020969	0.885424	0.033203	0.856121	0.000439	0.983371
2015	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	**F**	**p**
	Timing	1	52	0.222398	0.639192	0.137566	0.712219	0.400285	0.529715	1.111786	0.296569	1.351298	0.250359
	N level	1	52	0.404779	0.527422	2.201058	0.143952	0.795565	0.376532	4.122078	0.047456	4.219732	0.044999
	Method	1	52	3.187863	0.080019	2.201058	0.143952	3.120436	0.083185	0.869083	0.355518	0.659547	0.420422
	N level*Timing	1	52	0.112652	0.738495	1.238095	0.270956	0.202068	0.654925	0.807834	0.372905	3.712583	0.059476
	Method*Timing	1	52	0.366534	0.547533	0.137566	0.712219	0.093847	0.760566	0.044471	0.833803	1.426356	0.237780
	N level*Method	1	52	0.132725	0.717101	0.550265	0.461547	0.334669	0.565420	2.965164	0.091020	3.012718	0.088536
	N level*Method*Timing	1	52	0.512488	0.477266	1.238095	0.270956	0.074424	0.786082	1.078305	0.303882	0.213256	0.646153
2016	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p	F	p
	Timing	1	52	0.58676	0.447138	0.261745	0.611090	0.329549	0.568400	0.24263	0.624388	0.688716	0.410395
	N level	1	52	11.63410	0.001260	3.519016	0.066287	5.359576	0.024589	8.02188	0.006560	0.011924	0.913467
	Method	1	52	7.22661	0.009627	0.261745	0.611090	3.367669	0.072210	13.83039	0.000491	0.551355	0.461106
	N level*Timing	1	52	2.90852	0.094080	2.355705	0.130888	0.340442	0.562096	0.62113	0.434207	6.983032	0.010847
	Method*Timing	1	52	0.58676	0.447138	2.355705	0.130888	0.000797	0.977585	0.24263	0.624388	0.551355	0.461106
	N level*Method	1	52	2.81077	0.099636	2.355705	0.130888	1.939919	0.169604	0.10251	0.750121	1.774733	0.188607
	N level*Method*Timing	1	52	0.00334	0.954120	0.261745	0.611090	0.069435	0.793203	0.97051	0.329114	2.270761	0.137887

ANOVA analysis for treatment effects in Glasshouse Experiment 2

	2014				2015				2016			
Source	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p
PB	68.01	10.66	6.381	0.000001	1.392513	0.647282	2.151324	0.028869	4.250872	1.340942	3.170065	0.060000
NEPP	8.087	2.054	3.9376	0.000259	0.311385	0.094462	3.296417	0.910000	0.320000	0.075077	4.262295	0.320000
MEPP	4.950667	1.484538	3.334819	0.001227	1.033703	0.545178	1.896084	0.020000	4.098638	0.641791	6.386253	0.380000
GY	3.506622	0.812975	4.313318	0.010000	0.165990	0.288838	0.574683	0.050000	1.736341	0.581649	2.985202	0.030000
HI	0.002159	0.000627	3.445208	0.010000	0.005638	0.003126	1.803499	0.071999	0.005002	0.002217	2.256645	0.021795

APPENDIX 4: ANOVA for Chapter 7

SECTION A – FIELD EXPERIMENTS

ANOVA analysis for treatment effects at Roodebloem from 2014 to 2015

	Rood 2014				Rood 2015			
Source	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p
PFP_N	14.54501	2.836861	5.127148	0.010000	13.86399	2.005679	6.912366	0.010000
Grain N Uptake	11.49399	3.448856	3.332695	0.007179	6.270713	3.259152	1.924032	0.089653
Straw N Uptake	5.118955	3.997574	1.280515	0.010000	61.05953	28.17509	2.167146	0.056872
Total N Uptake	10.54402	4.166605	2.530603	0.029133	76.05609	37.04595	2.053020	0.070383
NHI	0.000453	0.000231	1.957873	0.010000	0.014296	0.004028	3.548672	0.750000

ANOVA analysis for treatment effects at Langgewens in 2015

	Lang 2015			
Source	MS Effect	MS Error	F	p
PFP_N	32.71950	9.156896	3.573209	0.030000
Grain N Uptake	32.12719	21.93907	1.464383	0.220995
Straw N Uptake	24.25550	1.264013	19.18928	0.010000
Total N Uptake	32.23432	17.62206	1.829203	0.030000
NHI	0.003772	0.001351	2.792416	0.022748
NUtE	4.899121	1.779430	2.753197	0.024255

ANOVA analysis for the method of N application, N rate and interactions at Roodebloem (2014 and 2015) and Langgewens (2015)

				Grain N uptake		Straw N Uptake		Total N uptake		N harvest index	
Rood 2014	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
	Method	1	20	0.002422	0.961239	3.651387	0.070464	2.177186	0.155641	3.64827	0.070574
	N Rate	1	20	1.109034	0.304850	2.286137	0.146175	0.484106	0.494575	3.60408	0.072164
	Method*N rate	1	20	9.800046	0.005267	4.705845	0.042286	0.059960	0.809054	16.38162	0.000630
Rood 2015	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
	Method	1	20	0.0698	0.794263	4.249307	0.052506	1.999379	0.172742	0.452130	0.509020
	N Rate	1	20	464.7603	0.000000	1.962568	0.176565	1.484674	0.237223	0.599909	0.447673
	Method*N rate	1	20	0.3881	0.540348	0.233502	0.634187	1.721652	0.204342	0.590051	0.451374
Lang 2015	Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
	Method	1	20	1.174774	0.291316	0.528011	0.475864	1.760101	0.199562	1.107869	0.305097
	N Rate	1	20	0.373310	0.548087	2.280589	0.146640	2.099554	0.162841	1.280089	0.271267
	Method*N rate	1	20	0.010440	0.919636	4.111330	0.056131	1.464836	0.240273	4.509371	0.046378

ANOVA analysis for the method of N application, N rate and interactions

				PFP_N		NUtE	
Rood 2014	Effect	Num. DF	Den. DF	F	p	F	p
	Method	1	20	0.7194	0.406362	2.401430	0.136904
	N Rate	1	20	153.7974	0.000000	1.251237	0.276572
	Method*N rate	1	20	6.9343	0.015934	6.161230	0.022056
Rood 2015	Effect	Num. DF	Den. DF	F	p	F	p
	Method	1	20	0.0698	0.794263	2.401430	0.136904
	N Rate	1	20	464.7603	0.000000	1.251237	0.276572
	Method*N rate	1	20	0.3881	0.540348	6.161230	0.022056
Lang 2015	Effect	Num. DF	Den. DF	F	p	F	p
	Method	1	20	1.06371	0.314681	1.002262	0.328723
	N Rate	1	20	13.60644	0.001455	0.605163	0.445720
	Method*N rate	1	20	0.04075	0.842059	3.309465	0.083887

SECTION B

ANOVA - GLASSHOUSE EXPERIMENT 1

ANOVA analysis for treatment effects in Glasshouse Experiment 1

	2013				2014				2016			
Source	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p	MS Effect	MS Error	F	p
PFP_N	13.46246	4.404718	3.056373	0.007812	31.17026	19.67575	1.584197	0.010000	63.63663	29.34425	2.168623	0.048339
Grain N Uptake	0.000120	0.000039	3.094341	0.004974	0.000398	0.000135	2.945709	0.006952	0.000414	0.000304	1.363808	0.010000
Straw N Uptake	0.000234	0.000043	5.425016	0.000035	0.000459	0.000253	1.812816	0.010000	0.000072	0.000031	2.280463	0.031372
Total N Uptake	0.000219	0.000074	2.946905	0.006934	0.000568	0.000353	1.609009	0.010000	0.000723	0.000434	1.665814	0.010000
NHI	0.000670	0.000668	1.003743	0.050000	0.002802	0.001377	2.034551	0.010000	0.000531	0.000743	0.714913	0.010000

ANOVA analysis for the method of N application, N rate and the interactions in Glasshouse Experiment 1

			Grain N Uptake		Straw N uptake		Total N uptake		NHI	
2013										
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
Method	1	44	196.2826	0.000000	30.88251	0.000002	201.9585	0.000000	0.497118	0.484485
N Rate	1	44	51.5478	0.000000	11.40899	0.001539	59.1979	0.000000	0.322253	0.573142
Method*N rate	1	44	4.7060	0.035502	2.59023	0.114677	7.7384	0.007931	0.303064	0.584752
2014										
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
Method	1	44	44.06329	0.000000	3.146776	0.082998	26.14235	0.000007	3.059413	0.087244
N Rate	1	44	6.69620	0.013041	2.787263	0.102117	6.59822	0.013677	0.182076	0.671676
Method*N rate	1	44	3.87658	0.055283	0.492491	0.486514	0.54896	0.462678	0.413799	0.523386
2016										
Effect	Num. DF	Den. DF	F	p	F	p	F	p	F	p
Method	1	44	3.785650	0.058099	0.02146	0.884207	2.876509	0.096946	2.43638	0.125714
N Rate	1	44	0.849770	0.361645	18.88004	0.000081	0.018273	0.893087	23.88390	0.000014
Method*N rate	1	44	2.124992	0.152015	4.04416	0.050476	0.728078	0.398128	7.24076	0.010036

ANOVA analysis for the time of N application, N rate, method of N application and interactions in Glasshouse Experiment 1

PFP_N				
2013				
Effect	Num. DF	Den. DF	F	p
N rate	1	44	3.58799	0.064784
Method	1	44	35.60179	0.000000
N rate*Method	1	44	0.00897	0.924975
2014				
Effect	Num. DF	Den. DF	F	p
N rate	1	44	44.06329	0.000000
Method	1	44	6.69620	0.013041
N rate*Method	1	44	3.87658	0.055283
2016				
Effect	Num. DF	Den. DF	F	p
N rate	1	44	5.214194	0.027288
Method	1	44	0.170125	0.682004
N rate*Method	1	44	4.267618	0.044765

SECTION C – GLASSHOUSE EXPERIMENT 2

ANOVA analysis for treatment effects at the Glasshouse Experiment 2 for 2014 and 2016

	2014				2016			
Source	**MS Effect**	**MS Error**	**F**	**p**	**MS Effect**	**MS Error**	**F**	**p**
PFP_N	179.8678	44.11402	4.077338	0.000309	70.77366	25.35381	2.791441	0.006891
Grain N Uptake	0.000634	0.000140	4.528853	0.000059	0.001084	0.000325	3.334677	0.001227
Straw N Uptake	0.000037	0.000019	1.929354	0.010000	0.000027	0.000012	2.201333	0.025266
Total N Uptake	0.000970	0.000169	5.746342	0.000003	0.001388	0.000304	4.563175	0.010000
NHI	0.001808	0.000543	3.330760	0.001240	0.001625	0.000611	2.657728	0.007429

ANOVA analysis for the time of N application, N rate, method of N application and interactions in Glasshouse Experiment 2

PFP$_N$				
2014				
Effect	Num. DF	Den. DF	F	p
Timing	1	52	0.47165	0.495279
N level	1	52	15.22638	0.000276
Method	1	52	14.90328	0.000315
N level*Timing	1	52	0.87704	0.353343
Method*Timing	1	52	0.53055	0.469641
N level*Method	1	52	0.20020	0.656415
N level*Method*Timing	1	52	1.31015	0.257610
2016				
Effect	Num. DF	Den. DF	F	p
Timing	1	52	0.47165	0.495279
N level	1	52	15.22638	0.000276
Method	1	52	14.90328	0.000315
N level*Timing	1	52	0.87704	0.353343
Method*Timing	1	52	0.53055	0.469641
N level*Method	1	52	0.20020	0.656415
N level*Method*Timing	1	52	1.31015	0.257610